A Phase II Neoadjuvant Study of Palbociclib in Combination with Letrozole and Trastuzumab as Neoadjuvant Treatment of Stage II-III ER+ HER2+ Breast Cancer (PALTAN)

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Protocol #: 201610019
Version Date: 11/13/2020

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Study Drug(s): Palbociclib (Ibrance)
Letrozole (Femara)
Trastuzumab (Herceptin)

IND #: 132365 EXEMPT
ClinicalTrials.gov #: NCT02907918

CONFIDENTIAL

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Protocol Revision History

Initial Approval Version	**12/23/16**
Amendment #1 Version	**03/10/17**
Amendment #2 Version	**09/20/17**
Amendment #3 Version	**11/07/17**
Amendment #4 Version	**08/09/18**
Amendment #5 Version	**10/25/19**
Amendment #6 Version	**11/13/2020**

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Principal Investigator Signature Page

Principal Investigator (printed):

Name of Institution:

PI Signature *Date*

By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB/HRPO procedures, the Declaration of Helsinki, ICH Good Clinical Practices guidelines, and the applicable parts of the United States Code of Federal Regulations or local regulations governing the conduct of clinical studies.

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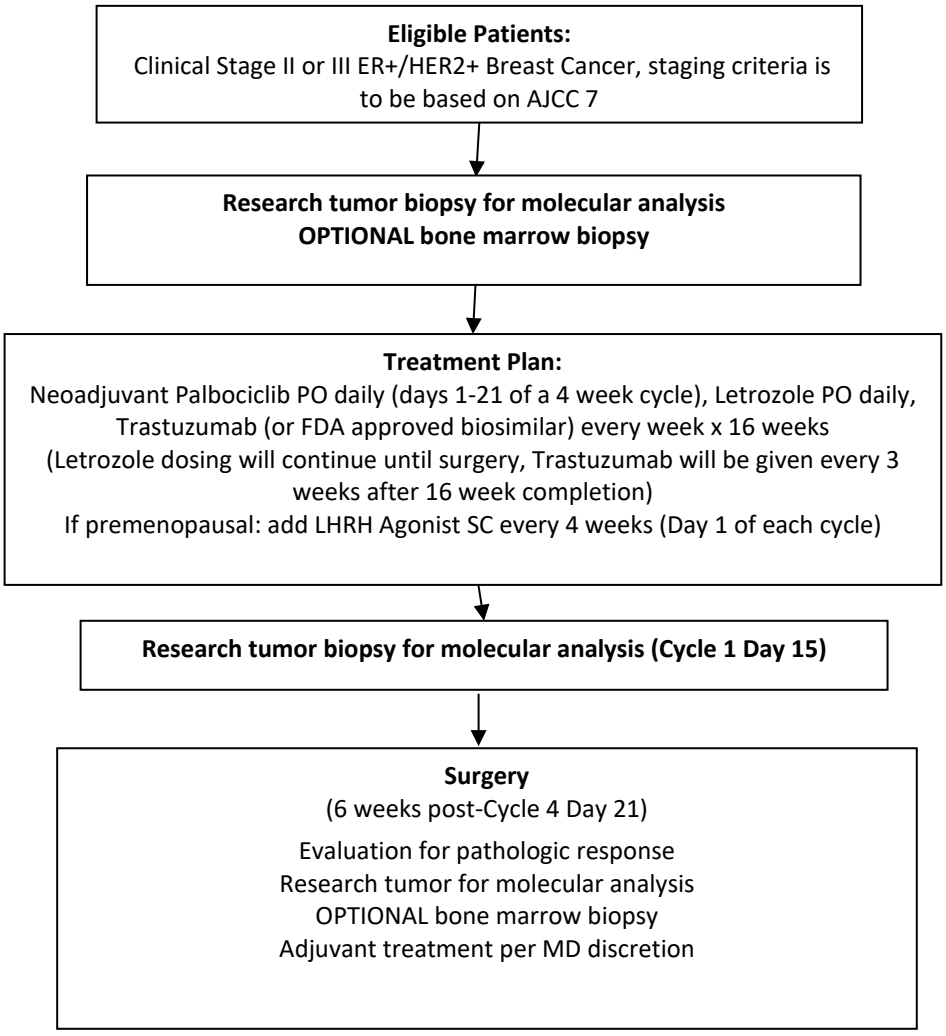
SCHEMA



Glossary of Abbreviations

AE	Adverse event
ALT (SGPT)	Alanine transaminase (serum glutamate pyruvic transaminase)
ANC	Absolute neutrophil count
AST (SGOT)	Aspartate transaminase (serum glutamic oxaloacetic transaminase)
AUC	Area under the curve
B-HCG	Beta human chorionic gonadotropin
BC	Breast cancer
BM	Bone marrow
BWFI	Bacteriostatic water for injection
CBC	Complete blood count
CFR	Code of Federal Regulations
CHF	Congestive heart failure
CR	Complete response
CRF	Case report form
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DNA	deoxyribonucleic acid
DSM	Data and Safety Monitoring
DSMC	Data Safety Monitoring Committee
DTC	Disseminated tumor cell
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EDTA	ethylenediaminetetraacetic acid
ER	Estrogen receptor
FDA	Food and Drug Administration
FFPE	Formalin-fixed paraffin-embedded
FSH	Follicle stimulating hormone
FWA	Federal wide assurance
GCP	Good Clinical Practice
GnRH	Gonadotropin releasing hormone
HHS	Department of Health and Human Services
HIV	Human Immunodeficiency Virus
HR	Hormone receptor
HRPO	Human Research Protection Office (IRB)
IB	Investigator brochure
IND	Investigational New Drug
IRB	Institutional Review Board
IULN	Institutional upper limit of normal
IV	Intravenous
IVPB	IV piggyback

LH	Luteinizing hormone
LVEF	Left ventricular ejection fraction
NC	No change
NCI	National Cancer Institute
NIH	National Institutes of Health
OCT	Optimum cutting temperature
OHRP	Office of Human Research Protections
pCR	Pathologic complete response
PD	Progressive disease
PFS	Progression-free survival
PI	Principal investigator
PO	Per os (by mouth)
PR	Partial response
QASMC	Quality Assurance and Safety Monitoring Committee
QD	Quaque die (one a day)
RNA	Ribonucleic acid
SAE	Serious adverse event
SC	Subcutaneous
SCC	Siteman Cancer Center
SWFI	Sterile water for injection
TTP	Time to progression
UPN	Unique patient number
WHO	World Health Organization
WUSM	Washington University School of Medicine

Table of Contents

1.0 BACKGROUND AND RATIONALE

1.1 HER2 Positive Breast Cancer

Breast cancer (BC) is the most common invasive cancer in women, with more than one million cases and over 411,000 deaths annually worldwide. Breast cancer is genetically heterogeneous and biologically diverse and is no longer considered a single disease. The long recognized clinical and phenotypic differences have been shown to correlate with differences at the gene expression level.[1,2] Five distinct molecular subtypes of BC are defined by hierarchical cluster analyses of array gene expression data and include luminal A, luminal B, HER2 amplified, basal-like and normal-like.[3] 20-25% of BC diagnoses are characterized by HER2 gene amplification or overexpression.[4-6] HER2 signaling drives cell proliferation and inhibits apoptosis in HER2+ BC.[4,6,7] Incorporating HER2-directed therapies is essential for the treatment of patients with HER2+ BC. However, despite available treatment options, over 10-20% of patients with HER2+ BC relapse with distant metastasis.

Approximately 50% of HER2+ BC is hormone receptor (HR) positive, a population that comprises about 10% of all BC patients.[8] Unlike the HR-/HER2+ tumors, which are HER2-E by molecular subtyping, HR+/HER2+ breast cancer has a high proportion in the luminal A and luminal B category.[9,10] Although the significance of these molecular features is to be further investigated, it is clear from previous neoadjuvant trials that these tumors are less likely to achieve a pathologic complete response (pCR) following neoadjuvant chemotherapy in combination with HER2 targeted agents, or to dual HER2 blockade.[10-12] In NeoSphere, therapy with neoadjuvant pertuzumab and trastuzumab with docetaxel resulted in improved pCR in patients with locally advanced BC.[11] However, fewer pCR rates were noted in HR+ versus HR- tumors (26.0% versus 63.2%, respectively). Similarly, the TRYPHAENA study, assessing both cardiac toxicity and pCR rate in patients with HER2+ BC undergoing neoadjuvant pertuzumab and trastuzumab with combinations of anthracycline- and non-anthracycline-based chemotherapy, found that the pCR rates were higher in patients with HR- tumors compared with HR+ tumors treated with combination of pertuzumab and trastuzumab.[12] The phase II CALGB 40601 study randomized nearly 300 patients to receive both trastuzumab and lapatinib, along with paclitaxel (THL), versus paclitaxel and trastuzumab (TH), versus paclitaxel and lapatinib (TL).[10] The pCR rate was similar between the THL and TH groups, 56% versus 46%, respectively ($p = 0.13$). Once again, there was no effect of dual therapy in the HR+ subset but a significant increase in pCR with dual therapy in HR- subset ($p = 0.01$). Furthermore, there was significant molecular heterogeneity using mRNA sequencing, with pCR rates significantly differing by intrinsic subtype (HER2 enriched, 70%; luminal A, 34%; luminal B, 36%; $p< 0.001$). These findings suggest that HR+/HER2+ tumors may be heavily influenced by ER signaling. Given this, it is of utmost importance to optimize selection of HER2 targeted treatment by identifying groups of patients not likely to benefit from dual HER2 blockade, both to minimize potential toxicities and improve cost-effectiveness.

'Cross-talk' between HER2 and ER pathways contributes to resistance to hormonal therapy in HR+/HER2+ BC. BC cell lines that are once sensitive to anti-estrogen therapy become

resistant once transfected with the HER-2/neu gene, suggesting an association between HER-2/neu overexpression and estrogen independence.[13] Trastuzumab restores tumor sensitivity to endocrine therapy also signifying a role for combination therapy compared to endocrine therapy alone. TAnDEM, a phase III trial in patients with HER2+/HR+ metastatic BC, demonstrated that the addition of trastuzumab to anastrozole improved time to disease progression (TTP) and progression free survival (PFS).[14] In the eLEcTRA trial, median TTP was 3.3 months in the letrozole alone group compared to 14.1 months in the letrozole plus trastuzumab group in HR+/HER2+ BC.[8] The phase II WSG-ADAPT trial assessed efficacy of 12 weeks of neoadjuvant TDM-1 or trastuzumab with endocrine therapy in HER2+/HR+ early BC in an attempt to determine responders of dual targeted therapy.[15] However the pCR rate was only 15.1% following neoadjuvant trastuzumab with endocrine therapy. Novel strategies are needed to improve the treatment efficacy for this patient population.

Dysregulation of cell cycle checkpoints is common in cancer, including BC. Preclinical data suggests that cyclin dependent kinases (CDK4/6) inhibition may play a key role in the treatment of subsets of BC. CDK4/6 in complex with cyclin D plays a critical role in G1 to S phase cell cycle progression through the regulation of the phosphorylation state of pRb.[16-18] Goel et al. recently reported that BC cells surviving HER2 withdrawal expressed high levels of cyclin D1 and CDK4 at time of recurrence in their transgenic mouse model[16]. Knockdown of cyclin D1 increased sensitivity of HER2-resistant cells to HER2 inhibition and a combined inhibition of CDK4/6 and HER2 resulted in synergistic reduction in cell proliferation in HER2 inhibitor–sensitive cells, thus restoring sensitivity to HER2 inhibition in previously resistant cells.

We propose to influence ER signaling by combining endocrine therapy with CDK4/6 inhibition along with trastuzumab in ER+/HER2+ early stage BC.

1.2 Palbociclib

1.2.1 Mechanism of Action

Palbociclib (PD 0332991) is a highly selective inhibitor of CDK4/cyclin D1 kinase activity. PD 0332991 has selectivity for CDK4/6, with little or no activity against a large panel of 34 other protein kinases including other CDKs and a wide variety of tyrosine and serine/threonine kinases. CDK6, another enzyme that also complexes with cyclin-D subunits, is also commonly expressed in mammalian cells and tumors. CDK6 is highly homologous to CDK4 and can perform the same function by phosphorylating Rb, thus potentially creating a redundant mechanism to promote cell cycle progression. Consequently, inhibition of both enzymes is necessary to ensure complete suppression of Rb phosphorylation and the greatest possible spectrum of antitumor activity. Results indicate that PD 0332991 inhibits CDK6 with equivalent potency to CDK4.

1.2.2 Non Clinical Studies

In vitro single-agent activity of PD 0332991

The only known natural substrate for CDK4/cyclinD1 is the retinoblastoma gene product (Rb). Specific CDK4 phosphorylation sites on Rb include serine-780 and serine-795. Therefore, the phosphorylation status of Rb at these specific sites in treated tumors can serve as an appropriate biomarker for target modulation by PD 0332991. The IC50 for reduction of Rb phosphorylation at serine-780 in the MDA-MB-435 breast carcinoma cell line was 0.066 μM. PD 0332991 was equally effective at reducing Rb phosphorylation at serine-795 in this tumor cell line with an IC50 of 0.063 μM. Similar effects on serine-780 and serine-795 Phosphorylation were obtained in the Colo-205 colon carcinoma cell line.

PD 0332991 inhibits cellular proliferation and prevents cellular DNA synthesis by preventing cells from entering S phase of the cell cycle. PD 0332991 inhibited thymidine incorporation into the DNA of a panel of Rb-positive human breast, colon, and lung carcinomas, with IC50 values ranging from 0.040 to 0.17 μM. PD 0332991 was also effective in preventing cell cycle progression in human leukemias and in non-transformed human epithelial cells and fibroblasts and was equally effective in suppressing cell division in human tumor cell lines. A selective CDK4/cyclin D inhibitor should cause a specific accumulation of cells in G1, but have no effect on other phases of the cell cycle, in which cells should continue to progress and eventually decline in number. MDA-MB-453 breast carcinoma cells that were exposed to various concentrations of PD 0332991 for 24 hours show a significant increase in the percentage of cells in G1 in the presence of as little as 0.04 μM PD 0332991 with a concomitant decline in other phases of the cell cycle.

Finally, to provide further evidence of the selectivity of PD 0332991, the compound was tested against Rb-negative tumor cells, which should not be sensitive to a specific Cdk4 inhibitor. PD 0332991 was tested against the MDA-MB-468 human breast carcinoma and the H2009 human non-small cell lung carcinoma, both of which have deleted Rb. The compound had no anti-proliferative activity on these cells when assayed at 3 μM (highest concentration tested), which is 1 to 2 orders of magnitude higher than the concentration necessary to inhibit Rb-positive tumor cells.

In Vivo Activity Studies

PD 0332991-0002 (hydrochloride salt) was used in all in vivo tumor models. Additionally, PD 0332991-0054 (isethionate salt) was used in the MDA-MB-435 breast carcinoma model, and had comparable efficacy to the hydrochloride salt.

The MTD in SCID mice was 150 mg/kg/day when administered orally, once a day, for 14 days. The MTD was defined as the highest dose that was nonlethal (<LD10). At the MTD on this regimen, PD 0332991 has significant antitumor efficacy against multiple human tumor xenograft models. The Colo-205 model is exquisitely sensitive to PD 0332991. At doses as low as 12.5 mg/kg, a 13-day growth delay

was obtained, indicating a 90% inhibition of tumor growth rate. PD 0332991 was inactive against the H23 lung and the SW-620 colon carcinomas. The lack of response may be associated with the presence of oncogenic K-ras mutations in SW-620 and H23; none of the xenografts sensitive to PD 0332991 had such mutations.

Further evidence that the anti-tumor activity observed in Rb-positive tumors is due to inhibition of CDK4/6 protein kinase activity was obtained by testing PD 0332991 in the MDA-MB-468 breast carcinoma and the DU-145 prostate tumor models. These are Rb negative tumors; neither of which responded to this compound. The lack of efficacy in Rb-negative tumors is consistent with the lack of anti-proliferative activity observed in vitro. Taken together, these results support the proposed mechanism of PD 0332991 (inhibition of CDK4/6-mediated Rb phosphorylation) and the specificity of the compound demonstrated in enzyme activity tests.

Further studies investigated whether continuous daily dosing of PD 0332991 was needed for optimal efficacy. Four dosing schedules were employed against the MDA-MB-435 breast carcinoma model over 14 days of treatment, including continuous daily, every other day, every third day, and 3 courses of 3 days dosing followed by 4-day drug holidays. The design of this experiment was such that the total compound administered over the 2-week period was identical for each treatment schedule. The results show that a similar degree of efficacy was attained with all schedules, implying that an intermittent regimen is feasible without compromising activity. Similar experiments were conducted against the Colo-205 colon carcinoma model. Again, intermittent schedules were as efficacious as daily dosing, with tumor regressions occurring during all dosing regimens.

During the 14-day treatment period employed for most of the efficacy experiments, no cures were documented, and the tumors grew back after therapy. It is possible that a tumor variant had selectively grown back and acquired resistance to the compound. To address this possibility, Colo-205 colon tumors that had initially significantly regressed in response to treatment with PD 0332991 were harvested and reimplanted into naive mice. After the tumors grew to 100 to 150 mg, these tumor-bearing mice were treated with PD 0332991 with a dose and dosing schedule identical to the original experiment. The tumors responded with equal sensitivity to the drug and fully regressed, indicating that no resistance had developed during the initial treatment. A similar result was observed with retreated MDAMB-435 tumors.

Pharmacokinetics

The single-dose pharmacokinetics of PD 0332991 following IV or PO routes of administration were investigated in Sprague-Dawley rats and Beagle dogs (toxicology species), and in cynomolgus monkeys. PD 0332991 was administered intravenously to determine elimination kinetics and absolute bioavailability from the PO route. Following IV administration, mean plasma clearance values of PD 0332991 in all species were low to moderate and were all lower than the corresponding hepatic blood flow. The mean apparent volumes of distribution at

steady state were approximately 10-fold greater than total body water. Mean absolute oral bioavailability of PD 0332991 was moderate in all species tested. In rats on Day 1 of repeat dose studies, mean PD 0332991 Cmax and AUC values increased in a dose-related manner up to 300 mg/kg. In dogs, mean PD 0332991 Cmax and AUC values increased in a dose-related manner up to 20 mg/kg on Day 1, and did not increase between 20 and 40 mg/kg. Mean PD 0332991 Cmax and AUC values in female rats were less than in male rats (up to one ninth and up to one sixteenth the values for male rats, respectively). There was no observed sex difference in systemic exposure in dogs. Mean PD 0332991 Cmax and AUC values following 3 weeks of dosing indicate up to 3-fold accumulation upon multiple dosing in both rats and dogs.

1.2.3 Clinical Development

As of 01 September 2015, 6 clinical studies in patients with palbociclib are ongoing and 3 clinical studies have completed. Additionally, as of 01 September 2015 palbociclib has been investigated in 16 completed and 4 ongoing Phase 1 clinical pharmacology studies in healthy volunteers. Four hundred seventy-three (473) healthy volunteers have received single doses of palbociclib ranging from 50 mg to 150 mg, and twenty-six healthy volunteers have received multiple doses of palbociclib on a 125 mg QD regimen.

1.2.4 Clinical Pharmacokinetics

To date pharmacokinetic data is available from four studies (see Section 8.1.3). The exposure (AUC(0-10) and Cmax) increased in a dose-proportional manner over the dose range of 25-225 mg QD following PD 0332991 administration on Days 1 and 8 of Cycle 1. At a steady state (Day 14 or Day 21), PD 0332991 was absorbed with a median Tmax of ~4 hours. PD 0332991 extensively penetrates into peripheral tissues, and was eliminated slowly; the mean elimination half-life (t1/2) was 26.5 hours.

The preliminary results from the recently performed food-effect study ("A5481021, a Phase 1, open-label 4 sequence 4 period crossover study of palbociclib (PD-0332991) in healthy volunteers to estimate the effect of food on the bioavailability of palbociclib") has provided evidence that when a single 125 mg dose of palbociclib was administered under fed conditions (including high fat or low fat meal given together with palbociclib, or moderate fat meal given 1 hour before and 2 hours after palbociclib) as a freebase formulation the palbociclib exposure levels were more uniform across the population than when taken in the fasting condition.

1.2.5 Clinical Toxicology

The most frequently reported AEs were predominantly considered treatment-related. These treatment-related events included fatigue, neutropenia, diarrhea, nausea, anemia, and thrombocytopenia. The most common Grade 4 adverse events were neutropenia and thrombocytopenia

QT Interval Effects
Data from non-clinical (in vitro and in vivo) studies indicated that PD 0332991 has the potential to delay cardiac repolarization as measured by prolongation of the QT interval on the ECG. Prolongation of QTcF (maximum increase of < 30 msec from baseline) was observed in a majority of patients in two Phase I trials with PD 0332991 as a single agent. No patient had a maximum QTcF value of > 500 msec during treatment. Notably, one female patient receiving PD 0332991 at 75 mg QD on Schedule 3/1 had a maximum QTcF increase of 67 msec from baseline to Cycle 1. Additionally, QTcF increases ranging from 39 to 51 msec compared to baseline persisted throughout her ECG collection period of 5 subsequent cycles. No significant changes in blood pressure, pulse rate and body weight have been observed in the two completed Phase 1 clinical studies in advanced cancers.

The patients enrolled in clinical studies should be closely monitored for potential cardiovascular symptoms. Appropriate monitoring should include clinical examinations, vital signs, routine ECGs, and AEs monitoring.

1.2.6 Developmental/Reproductive Toxicity

Fertility and teratology studies with PD 0332991 have not been conducted. Women of childbearing potential must have a negative pregnancy test prior to treatment with PD 0332991. Female patients must be surgically sterile or be postmenopausal, or must agree to use effective contraceptive during the period of the trial and for at least 90 days after completion of treatment.

1.3 Letrozole

Letrozole is indicated and approved for treating patients with HR+ breast cancers. Please refer to local prescribing information for key safety information.

1.4 Trastuzumab

Trastuzumab is indicated and approved for treating patients with HER2+ breast cancers. Signs and symptoms of cardiotoxicity and cardiac dysfunction have been observed in patients treated with trastuzumab, especially when used concurrently with anthracyclines. Please refer to local prescribing information for key safety information. Trastuzumab has available biosimilar products FDA approved for use. Trastuzumab or any of the FDA approved commercially available biosimilar products for trastuzumab may be administered to patients based on their insurance requirements or institutional practice.

1.5 Safety Data

Although there is an ongoing Spanish phase 2 study (NCT02448420) with this combination of palbociclib, letrozole and trastuzumab in the metastatic setting, no published data is available at this time, but per communication with Pfizer, the safety DMC review shows no concerning safety signal.

1.6 Correlative Studies Background

We plan to perform tumor biopsies to assess markers of tumor cell proliferation (Ki67), apoptosis (Cleaved PARP), and senescence (beta gal assay) following 2 weeks of combination therapy as early predictors of clinical and pathologic response. In addition, pharmacodynamic effect of palbociclib in combination with letrozole and trastuzumab on HER2 signaling (pHER2, pAKT, pMEK), ER (pER, PgR), CDK4/6-Rb (phosphor Rb $Ser^{801/811}$ and Ser^{780}) and cyclin D levels, and other relevant targets will be assessed by proteomics such as reverse phase protein array and immunohistochemistry analysis of tumors collected following 2 weeks of neoadjuvant therapy and at the time of surgery to compare with baseline.

Since PIK3CA mutation and PTEN status have been implicated in resistance mechanisms for HER2 targeted agents, tumor DNA at baseline and surgery time points will be subjected to next generation sequencing for whole exome (if funding permits) or targeted sequencing analysis.[18-23]

We plan to perform cDNA microarray analysis to assign intrinsic subtype using the PAM50 gene expression signature and to correlate with treatment response.[24] Previous studies indicated that ER+/HER2+ breast cancers are comprised of a mixture of luminal A, B and HER2-E tumors, which potentially affect their response to HER2-targeted agents.[25] There is no data in the literature indicating whether response to trastuzumab/letrozole/palbociclib combination differs according to molecular subtypes of breast cancer.

We will enrich for disseminated tumor cells (DTCs) and perform analysis for HER2 expression as well as expression of other genes associated with the metastatic potential of DTCs in pretreatment and post-treatment bone marrow; this is an optional collection and will be performed only in consenting patients. This analysis will lead to the identification of new predictive therapeutic markers which are associated with resistance to the combination of ER/HER2 targeted therapy. These genes will be compared with the expression profile of residual tumor to begin to assess the optimal predictors of recurrent disease development in this population of patients. We also examine the relationship between tumor subtype and DTC profile with the goal of understanding whether a specific tumor subtype is associated with HER2 positive DTCs.

2.0 OBJECTIVES

2.1 Primary Objective

To determine the pCR rate in patients with clinical stages II/III ER+ HER2+ breast cancer treated with neoadjuvant letrozole, trastuzumab (or FDA approved biosimilar), and palbociclib.

2.2 Secondary Objective

1. To assess safety and tolerability of palbociclib in combination with neoadjuvant letrozole and trastuzumab (or FDA approved biosimilar).
2. To assess the patient reported outcomes associated with palbociclib in combination with neoadjuvant letrozole and trastuzumab (or FDA approved biosimilar).

2.3 Exploratory Objectives

1. To determine if bone marrow disseminated tumor cells (DTC) status is a good indicator of clinical response and if subpopulations of DTCs emerge with neoadjuvant treatment.
2. To assess intrinsic breast cancer subtype utilizing cDNA microarray and to correlate with treatment response
3. To examine the pharmacodynamic effect of the study drug treatment by proteomic and immunohistochemistry analysis of baseline, 2-week and surgical tumor sample, and to correlate with treatment response
4. To examine the mutational profiles by whole exome or targeted gene sequencing at baseline and at time of definitive surgery to correlate with treatment response
5. To assess tumor cell apoptosis index post 2 weeks of palbociclib in combination with letrozole and trastuzumab (or FDA approved biosimilar).
6. To examine markers of cell proliferation, apoptosis and senescence on 2-week biopsies as predictors of surgical outcome.
7. To assess serum, plasma and ctDNA sequencing of selected genes, including ESR1, TP53, PIK3CA and RB, at baseline and surgery to correlate with response.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

1. Newly diagnosed clinical stage II or III ER+/HER2+ breast cancer with complete surgical excision of the breast cancer after neoadjuvant therapy as the treatment goal, staging criteria is to be based on AJCC 7.

2. Tumor size at least 2 cm in one dimension by clinical or radiographic exam (WHO criteria). Patients with histologically confirmed palpable lymph nodes may be enrolled regardless of breast tumor size. A palpable mass is not required as long as the mass is as least 2 cm in one dimension by radiographic exam.

3. At least 18 years of age.

4. ECOG performance status ≤ 1 (see Appendix A).

5. Normal bone marrow and organ function as defined below:
   a. Leukocytes ≥ 3,000/mcL
   b. Absolute neutrophil count ≥ 1,500/mcl
   c. Platelets ≥ 100,000/mcl
   d. Total bilirubin ≤ IULN
   e. AST(SGOT)/ALT(SGPT) ≤ 2.5 x IULN
   f. Creatinine ≤ IULN OR creatinine clearance ≥ 60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal

6. LVEF ≥ 50% by transthoracic echocardiogram or MUGA

7. Baseline corrected QT interval (QTcF) < 480 ms.

8. Women of childbearing potential must agree to undergo pregnancy testing within 14 days of study entry and agree to use adequate contraception (barrier method of birth control, abstinence, not hormonal) prior to study entry and for the duration of study participation as well as chemical LHRH Agonist with goserelin. Should a woman become pregnant or suspect she is pregnant while participating in this study, she must inform her treating physician immediately.

9. Ability to understand and willingness to sign an IRB approved written informed consent document (or that of legally authorized representative, if applicable).

3.2 Exclusion Criteria

1. Prior systemic therapy for indexed breast cancer.

2. Indeterminate or negative HER2 status.

3. Inflammatory breast cancer.

4. A history of other malignancy ≤ 5 years from diagnosis of indexed BC with the exception of basal cell or squamous cell carcinoma of the skin treated with local resection only or carcinoma *in situ* of the cervix.

5. Currently receiving any other investigational agents or received any within the past 28 days.

6. Know to be HIV positive.

7. Known hepatitis B or C infection.

8. A history of allergic reactions attributed to compounds of similar chemical or biologic composition to palbociclib, letrozole, trastuzumab, any other aromatase inhibitor, any other monoclonal antibody, or other agents used in the study.

9. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, or cardiac arrhythmia.

10. Current use or anticipated need for food or drugs that are known strong CYP3A4 inhibitors (i.e., grapefruit juice, verapamil, ketoconazole, miconazole, itraconazole, posaconazole, erythromycin, clarithromycin, telithromycin, indinavir, saquinavir, ritonavir, nelfinavir, lopinavir, atazanavir, amprenavir, fosamprenavir, nefazodone, diltiazem, and delavirdine) or inducers (i.e. dexamethasone, glucocorticoids, progesterone, rifampin, phenobarbital, St. John's wort).

11. Any condition that impairs the ability to swallow or absorb oral medication (e.g., gastrointestinal tract disease resulting in an inability to take oral medication or a requirement for IV alimentation, prior surgical procedures affective absorption).

12. Pregnant and/or breastfeeding. Women of childbearing potential must have a negative pregnancy test within 14 days of study entry.

3.3 Inclusion of Women and Minorities

Women and members of all races and ethnic groups are eligible for this trial.

4.0 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility by Washington University
2. Registration of patient in the Siteman Cancer Center database
3. Assignment of unique patient number (UPN)

Once the patient has been entered in the Siteman Cancer Center OnCore database, the WUSM coordinator will forward verification of enrollment and the UPN via email.

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility by collecting the information listed below and scanning and emailing it to the research coordinator listed in the *Siteman Cancer Center Clinical Trials*

Core Protocol Procedures for Secondary Sites packet at least one business day prior to registering patient:

1. Your name and contact information (telephone number, fax number, and email address)
2. Your site PI's name, the registering MD's name, and your institution name
3. Patient's race, sex, and DOB
4. Three letters (or two letters and a dash) for the patient's initials
5. Currently approved protocol version date
6. Copy of signed consent form (patient name may be blacked out)
7. Planned date of enrollment
8. Completed eligibility checklist, signed and dated by a member of the study team
9. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center OnCore Database

Registrations may be submitted Monday through Friday between 8am and 5pm CT. Urgent late afternoon or early morning enrollments should be planned in advance and coordinated with the Washington University research coordinator. Registration will be confirmed by the research coordinator or his/her delegate by email within one business day. Verification of eligibility and registration should be kept in the patient chart.

All patients at all sites must be registered through the Siteman Cancer Center OnCore database at Washington University.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. Patients will also be identified by first, middle, and last initials. If the patient has no middle initial, a dash will be used on the case report forms (CRFs). All data will be recorded with this identification number on the appropriate CRFs.

5.0 TREATMENT PLAN

Participants will be treated with neoadjuvant palbociclib in addition to letrozole (plus goserelin if premenopausal) and trastuzumab (or FDA approved biosimilar) for a total of 16 weeks, consisting of four 28-day cycles.

Definitive surgery will be performed preferably within 6 weeks after the end of Cycle 4 but may be delayed depending upon the availability of the surgeon. Letrozole and trastuzumab (or FDA approved biosimilar) will continue until the day of surgery. Letrozole will continue to be taken daily and trastuzumab (or FDA approved biosimilar) will be given every 3 weeks, per standard of care guidelines beginning the day after Cycle 4 Day 28. Adjuvant therapy following definitive surgery will be at the discretion of the treating physician.

5.1 Premedications

Patients will receive oral diphenhydramine at a dose of 50 mg and oral acetaminophen at a dose of 650 mg 60 minutes prior to the loading dose of trastuzumab (or FDA approved biosimilar), and on an as needed basis prior to any subsequent doses of trastuzumab (or FDA approved biosimilar).

5.2 Agent Administration

Palbociclib is an oral drug given at a dose of 125 mg daily on Days 1-21 of each 28-day cycle for a total of 4 cycles. Palbociclib should be taken with food. If a patient misses a day's dose entirely, she should not make the dose up the next day but just continue with the next day's regular dose. If a patient vomits any time after taking a dose, she should not re-take the dose but just continue to take subsequent doses as prescribed. If a patient inadvertently takes an extra dose during a day, she must be instructed to not take the next day's dose. Patients will be instructed to complete a drug diary (Appendix D) for compliance purposes.

Trastuzumab will be administered on a weekly basis for 16 weeks (on Days 1, 8, 15, and 22 of each 28-day cycle for a total of 4 cycles). Baseline weight is to be used for cycles 1-4. The first dose of trastuzumab on Cycle 1 Day 1 will be a loading dose of 4 mg/kg IVPB over 90 minutes. Subsequent doses of trastuzumab will be 2 mg/kg IVPB over 30 minutes. Trastuzumab will continue every 3 weeks after the completion of Cycle 4 of palbociclib until surgery beginning the day after Cycle 4 Day 28. Trastuzumab has available biosimilar products FDA approved for use. Trastuzumab or any of the FDA approved commercially available biosimilar products for trastuzumab may be administered to patients based on their insurance requirements or institutional practice. If a biosimilar product is required, dosing, schedule of administration, and premedications are identical to the guidance described throughout the protocol for trastuzumab. Preparation of the drug product will be dependent on the biosimilar selected for use.

Letrozole is an oral drug given at a dose of 2.5 mg orally once a day. It will be taken continuously (Days 1-28 of each cycle) until the day of definitive surgery. Letrozole may be taken with or without food. If a patient misses a day's dose entirely, she should not make the dose up the next day but just continue with the next day's regular dose. If a patient vomits any time after taking a dose, she should not re-take the dose but just continue to take subsequent doses as prescribed. If a patient inadvertently takes an extra dose during a day, she must be instructed to not take the next day's dose. Patients will be instructed to complete a drug diary (Appendix E) for compliance purposes. Letrozole will continue after the completion of Cycle 4 of palbociclib until the day of surgery.

Patients who are premenopausal will also be treated with goserelin as a means of LHRH Agonist. Goserelin is given subcutaneously at a dose of 3.6 mg on Day 1 of each cycle. Goserelin will be continued (once every 28-days) after the completion of Cycle 4 of palbociclib if required. Lupron (Leuprolide) may be substituted for Goserelin.

5.3 Post Surgery Therapy

Standard adjuvant treatment, including chemotherapy, antiHER2 therapy, hormonal therapy, and radiation (if needed), are recommended after surgery at the discretion of the treating physician.

5.4 Toxicity and Response Evaluations

All patients who receive any study treatment are evaluable for toxicity. Patients are evaluated from first receiving study treatment until a 30-day follow up after the conclusion of treatment or death. Patients who do not receive any study treatment will be replaced.

All patients are evaluable for disease response unless they discontinue treatment due to treatment related adverse events(s) prior to completion of Cycle 1.

Any patient that receives one dose on study is considered evaluable for toxicity. Patients are evaluated from first receiving study treatment until a 30-day follow up after the conclusion of treatment or death. Patients who do not receive any study treatment will be replaced.

Any patient that completes the first cycle of study treatment is considered evaluable for efficacy unless they discontinue treatment due to treatment related adverse events(s) prior to completion of Cycle 1.

5.5 Patient Reported Outcomes

Patient-reported outcomes will be evaluated using a modified PRO-CTCAE at baseline, after Cycle 1, and after completion of neoadjuvant therapy (Appendix F).

5.6 General Concomitant Medication and Supportive Care Guidelines

When taking palbociclib, patients should be instructed to avoid food or drugs that are known strong CYP3A4 inhibitors or inducers. Please refer to Appendix B for a list of prohibited medications.

No specific antidotes exist for the treatment of palbociclib overdose. Since renal excretion of palbociclib is minimal, the benefit of hemodialysis in the treatment of a palbociclib overdose is probably negligible. The treatment of overdose of palbociclib should consist of general supportive measures. If indicated, elimination of unabsorbed drug should be achieved by emesis or gastric lavage.

Guidelines for trastuzumab and letrozole will be as per standard of care. Use of estrogen containing oral contraceptives is prohibited as it will negate any benefit from letrozole.

5.7 Women of Childbearing Potential

Women of childbearing potential (defined as women with regular menses, women with amenorrhea, women with irregular cycles, women using a contraceptive method that precludes withdrawal bleeding, and women who have had a tubal ligation) are required to have a negative pregnancy test within 14 days prior to Cycle 1 Day 1.

Patients are required to use two forms of acceptable contraception, including one barrier method, during participation in the study and for 90 days following the last dose of palbociclib. Use of estrogen containing oral contraceptives is prohibited as it will negate any benefit from letrozole. Goserelin is not considered an acceptable form of contraception.

If a patient is suspected to be pregnant, study treatment should be immediately discontinued. In addition, a positive urine test must be confirmed by a serum pregnancy test. If it is confirmed that the patient is not pregnant, the patient may resume dosing.

If a patient becomes pregnant during therapy or within 28 days after the last dose of study treatment, the investigator must be notified in order to facilitate outcome follow-up.

5.8 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, treatment may continue for a maximum of 4 cycles or until one of the following criteria applies:

- Documented and confirmed disease progression
- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unable to receive further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious non-compliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar. Exception: Patients who progress on study, who do not go to surgery and initiate a new therapy, will be taken off study, but will continued to be followed according to section 5.9.

5.9 Duration of Follow-up

Patients will be observed post-surgery for 30-60 days or resolution of treatment-related adverse events, whichever comes later, then yearly for recurrence and survival status for 5 years. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

6.0 DOSE DELAYS/DOSE MODIFICATIONS

6.1 Re-Treatment Criteria

A new cycle of treatment with palbociclib may begin only if:

- ANC ≥ 1,000/mcL.
- Platelet count ≥ 50,000/mcL.
- Non-hematologic toxicities have returned to baseline or grade ≤1 severity (or, at the investigator's discretion, grade ≤2 if not considered a safety risk for the patient).

Criteria for dose interruption within cycle:

- ANC < 500/mcL.
- Platelet count < 50,000/mcL.

Re-treatment within the cycle may only be started when ANC ≥ 500/mcL and platelet count ≥ 50,000/mcL.

Doses omitted for toxicity within a cycle are not replaced or restored within the same cycle (meaning that the cycle remains 28 days regardless of the number of doses of taken).

If these conditions are not met, treatment with letrozole and trastuzumab may be continued but treatment with palbociclib must be delayed by one week. If, after a one-week delay, all toxicities have recovered within the limits described above, treatment with palbociclib can be resumed.

If the patient has not recovered after 2 weeks (excluding the scheduled one-week off treatment period within a cycle) despite dose reduction to the lowest dose level, treatment with palbociclib will be permanently discontinued.

6.2 Dose Modifications for Palbociclib

Dose Modification Table

Dose Level	Palbociclib Dose (Days 1-21 of each 28-day cycle)
1 (starting dose)	125 mg/day
-1	100 mg/day
-2	75 mg/day

Dose reduction below 75 mg/day is not allowed.

Patients will be monitored for toxicity and the dose of palbociclib may be adjusted as indicated in the table above. Dose reduction by 1, and if needed, 2 dose levels will be allowed depending on the type and severity of toxicity encountered (see table below). Patients requiring more than 2 dose reductions will be discontinued from the study.

Recommended dose reductions for palbociclib are detailed in the table below. Doses may be held as needed for toxicity resolution during a cycle. Doses omitted for toxicity are not replaced or restored within the same cycle (meaning that the cycle remains 28 days regardless of the number of doses of taken).

Palbociclib Dose Modifications Based on Worst Treatment-Related Toxicity in the Previous Cycle

Worst Toxicity During Previous Cycle	New Dose Level
Grade 4 neutropenia	Decrease by one dose level
Grade 4 thrombocytopenia	Decrease by one dose level
Grade 3 neutropenia associated with a documented infection or fever ≥ 38.5 ºC	Decrease by one dose level
Grade ≥ 3 non-hematologic toxicity (includes nausea, vomiting, diarrhea, and hypertension only if persisting despite maximal medical treatment)	Decrease by one dose level
Delay by > 1 week in receiving the next scheduled dose due to persisting treatment-related toxicities	If recovery occurs within 2 weeks, continue and decrease by one dose level
Inability to deliver at least 80% of the planned dose of palbociclib or letrozole due to adverse events possibly related to study treatment	Decrease by one dose level

6.2.1 Dose Adjustments Due to QTc Prolongation

Due to CYP3A inhibition mediated by palbociclib, the pharmacokinetics of drugs metabolized by CYP3A may be increased which, depending on the drug, may lead to QTc prolongation. In such situations, palbociclib should be discontinued at the physician's discretion, as should the drug responsible for QTc prolongation.

6.2.2 Dose Adjustments Due to Interstitial Lung Disease (ILD)/Pneumonitis

Monitor patients for pulmonary symptoms indicative of ILD/pneumonitis (e.g. hypoxia, cough, and dyspnea). In patients who have new or worsening respiratory symptoms and are suspected to have developed ILD/pneumonitis, interrupt palbociclib immediately and evaluate the patient. Permanently discontinue palbociclib in patients with severe ILD or pneumonitis.

6.3 Dose Modifications for Trastuzumab (or FDA Approved Biosimilar)

6.3.1 Infusion-associated Symptoms with Trastuzumab (or FDA Approved Biosimilar)

Infusion reactions consist of a symptom complex characterized by fever and chills, and on occasion nausea, vomiting, pain (in some cases at tumor sites), headache, dizziness, hypotension, rash, and asthenia. In post marketing reports, serious and fatal infusion reactions have been reported. Severe reactions which include bronchospasm, anaphylaxis, angioedema, hypoxia, and severe hypotension were usually reported during or immediately following the initial infusion. However, the onset and clinical course were variable including progressive worsening, initial improvement followed by clinical deterioration, or delayed post-infusion events with rapid clinical deterioration. For fatal events, death occurred within hours to days following a serious infusion reaction.

Interrupt trastuzumab (or FDA approved biosimilar) infusion in all patients experiencing dyspnea, clinically significant hypotension, and intervention of medical therapy administered, which may include: epinephrine, corticosteroids, antihistamines, including diphenhydramine, bronchodilators, and oxygen. Patients should be evaluated and carefully monitored until complete resolution of signs and symptoms. The rate of infusion should be decreased for mild or moderate (CTCAE 4.0 grade 1 or 2) infusion reactions. Permanent discontinuation should be strongly considered in all patients with severe (grade 3 or 4) infusion reactions.

6.3.2 Cardiac Dysfunction

Signs and symptoms of cardiac dysfunction were observed in a number of women who received trastuzumab alone or in combination with chemotherapy, most often anthracycline-based treatment. Cardiac dysfunction was observed most frequently among patients who received trastuzumab plus AC chemotherapy (28%), compared with those who received AC alone (7%), trastuzumab plus paclitaxel (11%), paclitaxel alone (1%), or trastuzumab alone (7%). Severe disability or fatal outcome due to cardiac dysfunction was observed in ~1% of all patients.

The nature of the observed cardiac dysfunction was similar to the syndrome of anthracycline-induced cardiomyopathy. The signs and symptoms of cardiac dysfunction usually responded to treatment. Complete and partial responses were observed among patients with cardiac dysfunction. The risk appears to be independent of tumor response to therapy. Analysis of the clinical database for predictors of cardiac dysfunction revealed only advanced age and exposure to an anthracycline as possible risk factors. In the clinical trials, most patients with cardiac dysfunction responded to appropriate medical therapy, often including discontinuation of trastuzumab. In many cases, patients were able to resume treatment with trastuzumab. In a subsequent study using weekly paclitaxel and trastuzumab as first-line treatment for metastatic breast cancer, the observed

incidence of serious cardiac dysfunction was 3% (N=95).[26] Since the occurrence of cardiac dysfunction in the trastuzumab plus chemotherapy trial was an unexpected observation, no information is available regarding the most appropriate method for monitoring cardiac function in patients receiving trastuzumab. Significant advances in the understanding and treatment of CHF have been made in the past several years, with many of the new drugs demonstrating the ability to normalize cardiac function. Patients who develop symptoms of congestive heart failure while on trastuzumab should be treated according to the HFSA guidelines.

All patients must have an evaluation of LVEF at baseline. After baseline, LVEF monitoring will be performed as per standard of care.

Recommended Cardiac Monitoring

Conduct thorough cardiac assessment, including history, physical examination, and determination of LVEF. The following schedule is recommended:

- Baseline LVEF measurement immediately prior to initiation of trastuzumab. Only patients with LVEF ≥ 50% by transthoracic echocardiogram or MUGA will be eligible.
- LVEF measurements every 3 months during and upon completion of trastuzumab using the same modality and same facility as used for baseline
- Repeat LVEF measurement at 4 week intervals if trastuzumab is withheld for significant left ventricular cardiac dysfunction

Asymptomatic Patients

If a patient does not have significant symptoms related to LV dysfunction, administration of trastuzumab will depend on the absolute change in LVEF between baseline and follow-up assessments.

Trastuzumab (or FDA approved biosimilar) should be initiated in an asymptomatic patient if:

- The LVEF increased or stayed the same;
- The LVEF decreased by ≤ 15 percentage points but is still at or above the lower limit of normal for the radiology facility.

Trastuzumab (or FDA approved biosimilar) is ***PROHIBITED*** in an asymptomatic patient if:

- The LVEF decreased ≤ 15 percentage points and is **below** the limit of normal for the radiology facility:
- The LVEF decreased by 16 percentage points or more (regardless of lower limits of normal for the radiology facility)
- The LVEF ≤ 50%

Withhold trastuzumab (or FDA approved biosimilar) dosing for at least 4 weeks for either of the following:

- The LVEF ≤ 50%
- > 16% absolute decrease in LVEF from pre-treatment values

- LVEF below institutional limits of normal and > 10% absolute decrease in LVEF from pretreatment values.
- Trastuzumab may be resumed if, within 4-8 weeks, the LVEF returns to normal limits and the absolute decrease from baseline is < 15%.
- Permanently discontinue trastuzumab for a persistent (> 8 weeks) LVEF decline or for suspension of trastuzumab dosing on more than 3 occasions for cardiomyopathy.

If a patient has significant symptoms related to left ventricular (LV) dysfunction, cardiac ischemia, or arrhythmia, initiation of trastuzumab (or FDA approved biosimilar) is prohibited.

6.4 Dose Modifications for Letrozole

No dose adjustment is permitted for letrozole, but interruptions no longer than 2 weeks will be allowed at the discretion of the treating physician.

7.0 REGULATORY AND REPORTING REQUIREMENTS

The entities providing oversight of safety and compliance with the protocol require reporting as outline below.

Patients will be observed post-surgery for 30-60 days or resolution of treatment-related adverse events, whichever comes later, then yearly for recurrence and survival status for 5 years. All adverse events must be recorded on the toxicity tracking case report from the (CRF) with the exception of:

- Baseline adverse events, which shall be recorded on the medical history CRF
-

Refer to the data submission schedule in Section 11.0 for instructions on the collection of AEs in the EDC.

Reporting requirements for Washington University study team may be found in Section 7.1. Reporting requirements for secondary site study teams participating in Washington University-coordinated research may be found in Section 7.2.

7.1 WU PI Reporting Requirements

7.1.1 Reporting to the Human Research Protection Office (HRPO) at Washington University

Reporting will be conducted in accordance with Washington University IRB Policies.

Pre-approval of all protocol exceptions must be obtained prior to implementing the change.

7.1.2 Reporting to the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The Washington University PI (or designee) is required to notify the QASMC of any unanticipated problem occurring at WU or any BJH or SLCH institution that has been reported to and acknowledged by HRPO. (Unanticipated problems reported to HRPO and withdrawn during the review process need not be reported to QASMC.)

QASMC must be notified within **10 days** of receipt of IRB acknowledgment via email to qasmc@wustl.edu. Submission to QASMC must include the myIRB forms and any supporting documentation sent with the form.

For events that occur at secondary sites, the Washington University PI (or designee) is required to notify the QASMC **within 10 days** of Washington University notification via email to qasmc@wustl.edu. Submission to QASMC must include either the myIRB form and supporting documentation or (if not submitted to myIRB) the date of occurrence, description of the event, whether the event is described in the currently IRB approved materials, the event outcome, determination of relatedness, whether currently enrolled participants will be notified, and whether the informed consent document and/or any study procedures will be modified as a result of this event.

7.1.3 Reporting to Secondary Sites

The Washington University PI (or designee) will notify the research team at each secondary site of all unanticipated problems involving risks to participants or others that have occurred at other sites within **10 working days** of the occurrence of the event or notification of the PI (or designee) of the event. This includes events that take place both at Washington University and at other secondary sites, if applicable. Refer to Section 16.0 (Multicenter Managament) for more information.

7.1.4 Reporting to Pfizer

Report any unexpected fatal or life-threatening adverse experiences (Appendix H, Section C.) associated with use of the drug or any serious, unexpected adverse experiences (Appendix H, Section D.), as well as results from animal studies that suggest significant clinical risk.

Within 24 hours of first awareness of the event (immediately if the event is fatal or life-threatening), the PI or designee will report to Pfizer by facsimile any serious adverse drug experience (as defined in Appendix H) that occurs during the SAE reporting period (as defined in Section 7.0) in a study subject assigned to receive palbociclib. Such SAEs will be reported using MedWatch form and the Pfizer Reportable Event Fax Cover Sheet (Appendix C) should also be included. SAEs should be reported as soon as they are determined to meet the definition, even if complete information is not yet available.

Even though there may not be an associated SAE, exposure to palbociclib during

pregnancy or lactation is reportable. In addition, occupational exposure to palbociclib is reportable, and a lack of effect of palbociclib may also be reportable.

Hy's Law Cases: Cases of potential drug-induced liver injury as assessed by laboratory test values ("Hy's Law Cases") are also reportable to Pfizer. If a study subject develops abnormal values in aspartate transaminase (AST) or alanine transaminase (ALT) or both, concurrent with abnormal elevations in total bilirubin and no other known cause of liver injury, that event would be classified as a Hy's Law Case.

Secondary sites must submit a completed MedWatch form to the Washington University PI and research coordinator within the specified time frame. The Washington University PI will be responsible for submitting all MedWatch forms from secondary sites to Pfizer.

7.1.5 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC. It is the responsibility of the Washington University principal investigator to report to the FDA as follows:

- Report any unexpected fatal or life-threatening suspected adverse reaction (refer to Appendix A for definitions) no later than 7 calendar days after initial receipt of the information.
- Report a suspected adverse reaction that is both serious and unexpected (SUSAR, refer to Appendix A) no later than 15 calendar days after it is determined that the information qualifies for reporting. Report an adverse event (refer to Appendix A) as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event, such as:
  - A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure
  - One or more occurrences of an event that is not commonly associated with drug exposure but is otherwise uncommon in the population exposed to the drug
  - An aggregate analysis of specific events observed in a clinical trial that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group
- Report any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies that suggest a significant risk in humans exposed to the drug no later than 15 calendar days after it is determined that the information qualifies for reporting.
- Report any findings from animal or in vitro testing that suggest significant risk in humans exposed to the drug no later than 15 calendar days after it is determined that the information qualifies for reporting.

- Report any clinically important increase in the rate of a serious suspected adverse reaction of that listed in the protocol or IB within 15 calendar days after it is determined that the information qualifies for reporting.

Submit each report as an IND safety report in a narrative format or on FDA Form 3500A or in an electronic format that FDA can process, review, and archive. Study teams must notify the Siteman Cancer Center Protocol Development team of each potentially reportable event within 1 business day after initial receipt of the information, and must bring the signed 1571 and FDA Form 3500A to the Siteman Cancer Center Protocol Development team no later than 1 business day prior to the due date for reporting to the FDA.

Each notification to FDA must bear prominent identification of its contents ("IND Safety Report") and must be transmitted to the review division in the Center for Drug Evaluation and Research (CDER) or in the Center for Biologics Evaluation and Research (CBER) that has responsibility for review of the IND. Relevant follow-up information to an IND safety report must be submitted as soon as the information is available and must be identified as such ("Follow-up IND Safety Report").

7.2 Secondary Sites Reporting Requirements

The research team at each secondary site is required to promptly notify the Washington University PI and designee of all serious adverse events (refer to Appendix A, Section D) within **1 working day** of the occurrence of the event or notification of the secondary site's PI of the event. This notification may take place via email if there is not yet enough information for a formal written report using an FDA Form 3500a (MedWatch)and Washington University'c cover sheet (Appendix C). A formal written report must be sent to the Washington University PI and designee within **4 calendar days** (for fatal or life-threatening suspected adverse reactions) or **11 calendar days** (for serious unexpected suspected adverse reactions) of the occurrence of the event or notification of the secondary site's PI of the event.

The research team at a secondary site is responsible for following its site's guidelines for reporting applicable events to its site's IRB according to its own institutional guidelines.

The research team at Washington University is responsible for reporting all applicable events to the FDA, and Pfizer as needed.

Washington University pre-approval of all protocol exceptions must be obtained prior to implementing the change. Local IRB approval must be obtained as per local guidelines. Washington University IRB approval is not required for protocol exceptions occurring at secondary sites.

7.3 Exceptions to Expedited Reporting

Events that do not require expedited reporting as described in Section 1.1 include:

- planned hospitalizations
- hospitalizations < 24 hours
- respite care
- events related to disease progression
- [study-specific events]

Events that do not require expedited reporting must still be captured in the EDC.

8.0 PHARMACEUTICAL INFORMATION

8.1 Palbociclib (Ibrance)

8.1.1 Palbociclib Description

Laboratory Code: PD 0332991-00
Molecular Weight: 447.54 Daltons
Molecular Formula: $C_{24}H_{29}N_7O_2$

Formulation: Capsules will be provided as the active ingredient with precedented excipients filled in hard gelatin capsules composed of gelatin and precedented colorants.

8.1.2 Clinical Pharmacology

Palbociclib is a highly selective inhibitor of CDK4/cyclinD1 kinase activity (IC_{50} = 11 nM; K_i = 2 nM). PD 0332991 has selectivity for CDK4/6, with little or no activity against a large panel of 34 other protein kinases including other CDKs and a wide variety of tyrosine and serine/threonine kinases. CDK6, another enzyme that also complexes with cyclin-D subunits, is also commonly expressed in mammalian cells and tumors. CDK6 is highly homologous to CDK4 and can perform the same function by phosphorylating Rb, thus potentially creating a redundant mechanism to promote cell cycle progression. Consequently, inhibition of both enzymes is necessary to ensure complete suppression of Rb phosphorylation and the greatest possible spectrum of antitumor activity. Results indicate that palbociclib inhibits CDK6 with equivalent potency to CDK4.

8.1.3 Pharmacokinetics and Drug Metabolism

As of 01 September 2015, twenty-seven clinical studies have evaluated the PK of palbociclib. Eight of these trials were conducted in patients with advanced malignant disease. Seventeen Phase 1 clinical pharmacology and biopharmaceutic studies of palbociclib were conducted in healthy subjects. Twelve of these 19 clinical trials were clinical pharmacology studies conducted to investigate the absorption, distribution, metabolism, and excretion of palbociclib as well as

examine the potential for DDI with palbociclib. The remaining 7 of the 17 clinical trials were biopharmaceutic studies conducted to examine the bioavailability, bioequivalence, and food effect of the palbociclib formulations.

Pharmacokinetic (PK) data from patients with advanced cancer from Study A5481001 indicate that the plasma pharmacokinetics of palbociclib are low to moderately variable with generally dose proportional exposures over the dose range evaluated (25 mg to 225 mg) following single and multiple doses. PK data from Studies A5481001, A5481003, and A5481010 indicate that palbociclib is slowly absorbed with a median time of maximum concentration (T_{max}) between 4 and 8 hours post-dose, and is slowly eliminated with an elimination half-life ($t_{1/2}$) ranging from 23.2 hours to 28.8 hours. Palbociclib accumulates after repeated daily dosing (median Rac ranged from 1.9 to 2.4), which was consistent with its terminal $t_{1/2}$. In Study A5481010, the median R_{ss} (the predicted accumulation to estimate linearity) was 1.1, indicating that palbociclib clearance does not change over time. In Study A5481003, palbociclib was shown to achieve steady-state concentrations following 8 days of QD dosing. The palbociclib geometric mean volume of distribution (V_z/F) was 2583 L in women with advanced breast cancer (Study A5481003), which is significantly greater than total body water (42 L), indicating that palbociclib extensively distributes to peripheral tissues.

In humans, metabolism is the major route of elimination of palbociclib. Following a single oral administration of [^{14}C]palbociclib to healthy subjects (Study A5481011), the overall median recovery of the administered radioactivity in the excreta was 91.6% with a median of 17.5% recovered in urine and a median of 74.1% recovered in feces. Excretion of unchanged palbociclib in the feces and urine was 2.3% and 6.9% of dose, respectively, indicating that excretion plays a minor role in elimination of palbociclib. A study in healthy volunteers (A5481015) indicated that the absolute oral bioavailability of palbociclib was approximately 46%.

In vitro data indicate that CYP3A and SULT enzyme SULT2A1 are mainly involved in the metabolism of palbociclib. Palbociclib is a weak time-dependent inhibitor of CYP3A following daily 125 mg dosing to steady state in humans. In vitro, palbociclib is not an inhibitor of CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, and 2D6, and is not an inducer of CYP1A2, 2B6, 2C8, and 3A4 at clinically relevant concentrations.

In vitro evaluations indicated that palbociclib has a low potential to inhibit the activities of drug transporters P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), organic anion transporter (OAT)1, OAT3, organic cation transporter (OCT)2, organic anion transporting polypeptide (OATP)1B1, OATP1B3, and bile salt export pump (BSEP) at clinically relevant concentrations.

An itraconazole DDI study in healthy volunteers (Study A5481016) and a rifampin DDI study in healthy volunteers (Study A5481017) were conducted to evaluate the

potential for strong CYP3A inhibitors and inducers, respectively, to alter the PK of palbociclib. Coadministration of itraconazole and palbociclib increased palbociclib AUC_{inf} and C_{max} by approximately 87% and 34%, respectively, relative to those when palbociclib dose was given alone. Coadministration of rifampin and palbociclib decreased palbociclib AUC_{inf} and C_{max} by approximately 85% and 70%, respectively, relative to palbociclib given alone. Based on this data, the concurrent administration of strong CYP3A inhibitors and inducers with palbociclib should be avoided.

A midazolam DDI study in healthy volunteers (Study A5481012) was conducted to evaluate the potential for palbociclib to act as a time-dependent inhibitor of CYP3A4/5 at steady-state. Plasma midazolam C_{max} and AUC_{inf} values increased 37% and 61%, respectively, when single oral doses of midazolam were co-administered with multiple doses of palbociclib as compared to its administration alone. This is consistent with weak time-dependent CYP3A4/5 inhibition mediated by palbociclib at steady-state following daily 125 mg dosing.

PK data from the Phase 1 portion of Study A5481003 was analyzed to evaluate the potential for a drug-drug interaction (DDI) between palbociclib and letrozole at steady-state. These data indicate a lack of a potential for DDIs between palbociclib and letrozole when administered in combination.

Data from a DDI study in healthy male subjects indicated that palbociclib exposures were comparable when a single dose of palbociclib was coadministered with multiple doses of tamoxifen and when palbociclib was given alone.

The effect of food on the exposure of palbociclib when administered as the commercial free base capsule was evaluated in healthy subjects (A5481021). Compared to palbociclib given under overnight fasted conditions, the AUC_{inf} and C_{max} of palbociclib increased by 21% and 38% when given with high-fat food, by 12% and 27% when given with low-fat food, and by 13% and 24% when moderate-fat food was given 1 hour before and 2 hours after palbociclib dosing. In addition, food intake significantly reduced the intersubject and intrasubject variability of palbociclib exposure. Based on these results, palbociclib commercial free base capsules should be taken with food.

The solubility of the palbociclib free base is pH dependent—palbociclib is water soluble at low pH (2.1-4.5), while the solubility dramatically decreases as pH rises above 4.5. Concomitant administration of agents which increase gastric pH can alter the solubility and absorption of palbociclib free base formulations.

In a drug interaction trial in healthy subjects (A5481038), coadministration of a single 125 mg dose of commercial free base capsule with multiple doses of the proton pump inhibitors (PPI) rabeprazole under fed conditions decreased palbociclib C_{max} by 41%, but had limited impact on AUC_{inf} (13% decrease), when compared to a single dose of palbociclib administered alone. Given the reduced

effect on gastric pH of H2-receptor antagonists and local antacids compared to PPIs, the effect of these classes of acid-reducing agents on palbociclib exposure under fed conditions is expected to be minimal. Under fed conditions there is no clinically relevant effect of PPIs, H2-receptor antagonists, or local antacids on palbociclib exposure. In another healthy subject study, coadministration of a single dose of commercial free base capsule with multiple doses of the PPI rabeprazole under fasted conditions decreased palbociclib AUC_{inf} and C_{max} by 62% and 80%, respectively, when compared to a single dose of palbociclib administered alone. Collectively, these antacid DDI data further support the requirement that the free base capsule of palbociclib should be taken with food.

Based on a population pharmacokinetic analysis in 183 patients with cancer (50 male and 133 female patients, age range from 22 to 89 years, and body weight range from 37.9 to 123 kg), gender had no effect on the exposure of palbociclib, and age and body weight had no clinically important effect on the exposure of palbociclib.

Based on a population pharmacokinetic analysis that included 183 patients, where 40 patients had mild hepatic impairment (total bilirubin ≤ ULN and AST > ULN, or total bilirubin >1.0 to 1.5 × ULN and any AST), mild hepatic impairment had no effect on the exposure of palbociclib. The pharmacokinetics of palbociclib have not been studied in patients with moderate or severe hepatic impairment (total bilirubin >1.5 × ULN and any AST).

Based on a population pharmacokinetic analysis that included 183 patients, where 73 patients had mild renal impairment (60 mL/min ≤ CrCl <90 mL/min) and 29 patients had moderate renal impairment (30 mL/min ≤ CrCl <60 mL/min), mild and moderate renal impairment had no effect on the exposure of palbociclib. The pharmacokinetics of palbociclib have not been studied in patients with severe renal impairment.

A pharmacokinetic/pharmacodynamic analysis to evaluate the relationship between palbociclib exposure and ECG endpoints (RR and QTc intervals) were developed using pooled data from 3 clinical trials in patients with advanced malignant disease (Studies A5481001, A5481002, and A5481003). The study population consisted of 48 men and 136 women with a median (range) body weight of 73.0 (37.9-123) kg and age of 61.5 (22-89) years old. Palbociclib doses ranged from 25 mg to 225 mg QD. The data collected from 184 patients consisted of 569 ECG-palbociclib concentration-matched pairs; the observed plasma concentrations had a median (range) of 55.2 (2.51-329) ng/mL. The average heart rate, RR, QT, QT corrected for heart rate according to Bazett (QTcB), QT corrected for heart rate according to Fridericia (QTcF), and QTcS (QT interval corrected for heart rate according to a study-specific correction factor) at baseline for ECG-palbociclib concentration matched data were 76.8 beats per minute, 808 msec, 380 msec, 425 msec, 409 msec, and 412 msec, respectively.

The results of the analysis indicate that palbociclib does not appear to have a concentration-dependent effect on heart rate. A slight positive linear relationship between palbociclib concentration and QTcS was observed; however, at the mean or median steady-state palbociclib C_{max} following administration of the recommended clinical dose of palbociclib (125 mg QD) in patients with cancer, the upper bound of the one-sided 95% CI for the increase in QTcS fell below the threshold of 10 msec, suggesting that QT prolongation is not a safety concern for palbociclib at the recommended clinical dose according to the criteria described in the ICH guidance for Industry E14. Similar results were obtained when QTcF and QTcB were used.

8.1.4 Supplier(s)

Pfizer will supply the study agent. The study agent will be free of charge to the patient.

8.1.5 Dosage Form and Preparation

Medication will be provided in non-patient specific bottles containing either 75 mg, 100 mg, or 125 mg capsules. The patient number and the protocol number should be recorded on the bottle label in the spaces provided. Site personnel must ensure that patients clearly understand the directions for self-medication. Patients should be given a sufficient supply to last until their next study visit. Unused drug and/or empty bottles should be returned to the site at the next study visit. Palbociclib is an agent that must be handled and administered with care. Patients should be instructed to keep their medication in the bottles provided and not transfer it to any other container. Due to possible unknown hazards associated with topical and environmental exposure to experimental agents, capsules must not be opened and/or emptied into any vehicle for oral ingestion; capsules must be swallowed intact.

8.1.6 Storage and Stability

Palbociclib capsules should be stored at controlled room temperature (20-25°C, 68-77°F) in their original container. Medication should be kept in a secured locked area at the study site in accordance with applicable regulatory requirements. Returned medication should be stored separately from medication that needs to be dispensed.

All containers of palbociclib that were sent to the investigator throughout the study must be returned to the sponsor or designee, whether they are used or unused, and whether they are empty or contain capsules.

8.1.7 Administration

Patients should be instructed to swallow palbociclib capsules whole and not to chew

them prior to swallowing. No capsule should be ingested if it is broken, cracked, or otherwise not intact. Patients should take palbociclib with food and should be encouraged to take their dose at approximately the same time each day.

8.1.8 Special Handling Instructions

No special handling instructions.

8.1.9 Pregnancy

The nonclinical safety profile of palbociclib has been well characterized through the conduct of single- and repeat-dose toxicity studies up to 39 weeks in duration, and safety pharmacology, genetic toxicity, reproductive and developmental toxicity, and phototoxicity studies. Consistent with the pharmacologic activity of palbociclib (cell cycle inhibition, CDK4/6 inhibition), the primary target organ findings included hematolymphopoietic (decreased cellularity of bone marrow and lymphoid organs) and male reproductive organ (seminiferous tubule degeneration, and secondary effects on the epididymis, prostate, and seminal vesicle) effects in rats and dogs, and altered glucose metabolism that was accompanied by effects on the pancreas and secondary changes in the eye, teeth, kidney, and adipose tissue in rats only, and effects on bone in rats only that were observed following single and/or repeat dosing at clinically relevant exposures. Altered glucose metabolism (hyperglycemia/glucosuria) correlated with pancreatic islet cell vacuolation that was determined to reflect a loss of beta cells with corresponding decreases in insulin and C-peptide. The reversibility of the effects on glucose homeostasis, pancreas, eye, kidney, and bone was not established following a 12-week non-dosing period; whereas partial to full reversal of effects on the hematolymphopoietic and male reproductive systems, teeth, and adipose tissue were observed. Additionally, a potential for QTc prolongation and hemodynamic effects were identified from safety pharmacology studies, and developmental toxicity was identified from embryo-fetal development studies in the rat and rabbit. Though gastrointestinal effects would be anticipated from a cell cycle inhibitor and while effects were observed in rats and dogs following single- and repeat-dose studies up to 3 weeks in duration (emesis, fecal changes, and microscopic changes in stomach and intestines), the effects were of limited severity at clinically relevant doses. Gastrointestinal effects were not prominent in longer duration studies, limited to effects on the glandular stomach and rodent-specific effects on the non-glandular stomach in rats following 27 weeks of intermittent dosing that did not reverse during a 12-week non-dosing period. Additional palbociclib-related findings considered non-adverse at tolerated doses based on limited severity and/or absence of degenerative changes included cellular vacuolation in multiple tissues that was morphologically consistent with phospholipidosis; hepatic (increases in liver enzymes, hepatocellular hypertrophy/increased vacuolation), renal (increased CPN), adrenal (cortical cell hypertrophy), and respiratory (clinical signs, tracheal epithelial cell atrophy) effects; and prolonged coagulation times. Reversibility

(partial or full) was established for these additional toxicities. Finally, palbociclib was determined to be an aneugen, for which a no effect exposure was identified

8.1.10 QT Interval

Palbociclib does not appear to have a concentration-dependent effect on heart rate. A slight positive linear relationship between palbociclib concentration and QTcS was observed; however, at the mean or median steady-state palbociclib Cmax following administration of the recommended clinical dose of palbociclib (125 mg QD) in patients with cancer, the upper bound of the one-sided 95% CI for the increase in QTcS fell below the threshold of 10 msec, suggesting that QT prolongation is not a safety concern for palbociclib at the recommended clinical dose according to the criteria described in the ICH guidance for Industry E14.

8.2 Trastuzumab (Herceptin)

8.2.1 Description

Trastuzumab (Herceptin) is a recombinant DNA-derived humanized monoclonal antibody that selectively binds with high affinity to the extracellular domain of HER2 (Kd = 5 nM).[27,28] The antibody is an IgG_1 kappa that contains human framework regions with the complementarity-determining regions of a murine antibody (4D5) that binds to HER2.

8.2.2 Use of FDA Approved Biosimilars

Trastuzumab has available biosimilar products FDA approved for use. Trastuzumab or any of the FDA approved commercially available biosimilar products for trastuzumab may be administered to patients based on their insurance requirements or institutional practice. If a biosimilar product is required, dosing, schedule of administration, and premedications are identical to the guidance described throughout the protocol for trastuzumab. Preparation of the drug product will be dependent on the biosimilar selected for use.

8.2.3 Pharmacokinetics and Drug Metabolism

Trastuzumab administered once weekly demonstrated dose-dependent pharmacokinetics. Mean half-life increased and clearance decreased with increasing dose level. The half-life averaged 1.7 and 12 days at the 10 and 500 mg dose levels, respectively. Trastuzumab's volume of distribution was approximately that of serum volume (44 mL/kg). At the highest weekly dose studied (500 mg), mean peak serum concentrations were 377 mcg/mL.

In studies using a loading dose of 4 mg/kg followed by a weekly maintenance dose of 2 mg/kg, a mean half-life of 5.8 days (range = 1 to 32 days) was observed. Between Weeks 16 and 32, trastuzumab serum concentrations reached a steady state with a mean trough and peak concentrations of approximately 79 microgram/mL and 123 microgram/mL, respectively.

Data suggest that the disposition of trastuzumab is not altered based on age or serum creatinine (up to 2.0 mg/dL). No formal interaction studies have been performed.

8.2.4 Supplier(s)

Trastuzumab is commercially available.

8.2.5 Dosage Form and Preparation

Trastuzumab is a sterile, white to pale yellow, preservative-free lyophilized powder for intravenous (IV) administration. Each 150mg trastuzumab vial for injection is supplied in a single-dose vial as a lyophilized sterile powder under vacuum.

Use appropriate aseptic technique. Each 150 mg vial of trastuzumab should be reconstituted with 7.4 ml of SWFI (not supplied) to yield a solution for single-dose use, containing 21mg/ml of trastuzumab.

Determine the dose of trastuzumab needed. Calculate the correct dose using 21 mg/mL trastuzumab solution. Withdraw this amount from the vial and add it to an infusion bag containing 250 mL of 0.9% sodium chloride, USP. **DEXTROSE (5%) SOLUTION SHOULD NOT BE USED.** Gently invert the bag to mix the solution. The reconstituted preparation results in a colorless to pale yellow transparent solution. Parenteral drug products should be inspected visually for particulates and discoloration prior to administration.

No incompatibilities between trastuzumab and polyvinylchloride or polyethylene bags have been observed.

8.2.6 Storage and Stability

Vials of trastuzumab are stable at 2-8°C (36-46°F) prior to reconstitution. Do not use beyond the expiration date stamped on the vial. A vial of 150mg trastuzumab reconstituted with SWFI contains no preservative and is intended for single-use only. The reconstituted trastuzumab solution should be used immediately and any unused portion must be discarded. DO NOT FREEZE TRASTUZUMAB THAT HAS BEEN RECONSTITUTED.

The solution of trastuzumab for infusion diluted in polyvinylchloride or polyethylene bags containing 0.9% Sodium Chloride Injection, USP, may be stored at 2-8°C (36-46°F) for up to 24 hours prior to use. Discard after 24 hours.

8.2.7 Administration

Please refer to Section 5.1.

8.2.8 Special Handling Instructions

None.

8.3 Letrozole (Femara)

8.3.1 Letrozole Description

Letrozole is indicated for:

- the adjuvant treatment of postmenopausal women with hormone receptor positive early breast cancer;
- the extended adjuvant treatment of early breast cancer in postmenopausal women who have received 5 years of adjuvant tamoxifen therapy;
- first-line treatment of post-menopausal women with hormone receptor positive or unknown, locally advanced or metastatic breast cancer;
- the treatment of advanced breast cancer in postmenopausal women with disease progression following antiestrogen therapy.

Letrozole is a nonsteroidal aromatase inhibitor (inhibitor of estrogen synthesis). It is chemically described as 4,4'-(1H-1,2,4-Triazol-1-ylmethylene)dibenzonitrile. It has a molecular weight of 285.31 and an empirical formula of $C_{17}H_{11}N5$.

8.3.2 Clinical Pharmacology

The growth of some cancers of the breast is stimulated or maintained by estrogens. Treatment of breast cancer thought to be hormonally responsive (i.e., estrogen and/or progesterone receptor positive or receptor unknown) has included a variety of efforts to decrease estrogen levels (ovariectomy, adrenalectomy, hypophysectomy) or inhibit estrogen effects (antiestrogens and progestational agents). These interventions lead to decreased tumor mass or delayed progression of tumor growth in some women.

In postmenopausal women, estrogens are mainly derived from the action of the aromatase enzyme, which converts adrenal androgens (primarily androstenedione and testosterone) to estrone and estradiol. The suppression of estrogen biosynthesis in peripheral tissues and in the cancer tissue itself can therefore be achieved by specifically inhibiting the aromatase enzyme.

Letrozole is a nonsteroidal competitive inhibitor of the aromatase enzyme system; it inhibits the conversion of androgens to estrogens. In adult nontumor- and tumor-bearing female animals, letrozole is as effective as ovariectomy in reducing uterine weight, elevating serum LH, and causing the regression of estrogen-dependent tumors. In contrast to ovariectomy, treatment with letrozole does not lead to an increase in serum FSH. Letrozole selectively inhibits gonadal steroidogenesis but has no significant effect on adrenal mineralocorticoid or glucocorticoid synthesis.

Letrozole inhibits the aromatase enzyme by competitively binding to the heme of the cytochrome P450 subunit of the enzyme, resulting in a reduction of estrogen biosynthesis in all tissues. Treatment of women with letrozole significantly lowers serum estrone, estradiol and estrone sulfate and has not been shown to significantly affect adrenal corticosteroid synthesis, aldosterone synthesis, or synthesis of thyroid hormones.

8.3.3 Pharmacokinetics and Drug Metabolism

Letrozole is rapidly and complete absorbed from the gastrointestinal tract and absorption is not affected by food. About 90% of letrozole is recovered in urine. Its terminal elimination half-life is about 2 days and steady-state plasma concentration after daily 2.5 mg dosing is reached in 2-6 weeks.

8.3.4 Supplier(s)

Letrozole is commercially available.

8.3.5 Dosage Form and Preparation

Letrozole is available as 2.5 mg tablets.

8.3.6 Storage and Stability

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

8.3.7 Administration

Please refer to Section 5.1.

8.3.8 Special Handling Instructions

None.

8.4 Goserelin (Zoladex)

8.4.1 Goserelin Description

Goserelin is a gonadotropin releasing hormone (GnRH) agonist indicated for:

- Use in combination with flutamide for the management of locally confined carcinoma of the prostate
- Palliative treatment of advanced carcinoma of the prostate
- The management of endometriosis
- Use as an endometrial-thinning agent prior to endometrial ablation for dysfunctional uterine bleeding

- Use in the palliative treatment of advanced breast cancer in pre- and peri-menopausal women

Goserelin acetate is chemically described as an acetate salt of [D-Ser(Bu^t)^6,Azgly^{10}]. Its chemical structure is pyro-Glu-His-Trp-Ser-Tyr-D-Ser(Bu^t)-Leu-Arg-Pro-Azgly-NH_2 acetate [$C_{59}H_{84}N_{18}O_{14}\cdot(C_2H_4O_2)_x$ where x = 1 to 2.4].

8.4.2 Clinical Pharmacology

Goserelin is a synthetic decapeptide analogue of GnRH. Goserelin acts as an inhibitor of pituitary gonadotropin secretion when administered in the biodegradable formulation.

8.4.3 Supplier(s)

Goserelin is commercially available.

8.4.4 Dosage Form and Preparation

Goserelin is available in two dosages. At a dose of 10.8 mg, it is administered subcutaneously every 12 weeks into the anterior abdominal wall. At a dose of 3.6 mg, it is administered every 28 days.

8.4.5 Storage and Stability

Do not store above 25°C.

8.4.6 Administration

Please refer to Section 5.1

8.4.7 Special Handling Instructions

None.

8.5 Lupron (Leuprolide)

8.5.1 Lupron Description

Lupron may be substituted for Goserelin. It is a synthetic gonadotropin-releasing hormone indicated for:

- Use in combination with flutamide for the management of locally confined carcinoma of the prostate
- Palliative treatment of advanced carcinoma of the prostate

- The management of endometriosis
- Use as an endometrial-thinning agent prior to endometrial ablation for dysfunctional uterine bleeding
- Use in the palliative treatment of advanced breast cancer in pre- and peri-menopausal women

9.0 CORRELATIVE STUDIES

9.1 Archival Tumor Sample Submission

Ten of 5 micron section unstained slides from the pre-treatment archival diagnostic core biopsy of the primary breast cancer will be requested for correlative studies that investigate predictors of response and mechanisms of action of the study treatment. Alternatively, a tumor block could be submitted, which will be returned after sectioning.

All samples should be marked with the patients' study number, initials and date of the sample using an indelible marker. Please include the pathology report associated with the archival tumor material.

Archival tumor specimens along with the completed Archival Tumor Specimen Submission Form (Appendix G) are to be shipped to Dr. Cynthia Ma's laboratory at the address below:

Dr. Cynthia Ma Laboratory
Attn: Jeremy Hoog
Washington University School of Medicine
4515 McKinley Research Building
Campus Box 8076
3rd Floor, Room 3111A
St. Louis, MO 63110
Phone: (314) 747-9309

9.2 Tumor Biopsy and Peripheral Blood Collection

9.2.1 Collection of Specimen(s)

The kit is a two-chamber kit in which it is possible to send both frozen and ambient specimens. The kit is stocked with all the necessary items needed to draw blood and obtain tissue specimens. It also contains the necessary materials used to process and prepare specimens for shipment in accordance to IATA regulations. Finally the kit contains all required documentation and labels required to return the kit to the Washington University Tissue Procurement Core Facility (address listed below).

It is advised that the baseline samples are harvested during port placement to optimize tissue acquisition.

Please note, secondary sites will not be submitting PDX samples.

Correlative study	Blood/tumor	Type of tube	Volume	Time point	Process at site	Temperature conditions for storage/shipping
Archival Tumor	Tumor rich block/slides	N/A	N/A	Diagnostic, Surgery	Yes	Blocks/slides can be shipped at room temperature same day or overnight
Tumor for research	Core biopsies	1 core snap frozen, 2 cores preserved OCT, 1 core PDX (no C1D15), 1 core 10% formalin (C1D15 only)	14G core needle	Baseline, C1D15, Surgery	No	Cores should be immediately snap frozen and ship on dry ice; cores in formalin should be stored / shipped at ambient temperature same day or overnight (Avoid Fridays).
Plasma for research	Whole blood	EDTA (purple)	10mL	Baseline, C1D15, Surgery, Yearly post-surgery for 5 years	Yes	-80ºC
Serum for research	Whole blood	Clot tube (red)	10mL	Baseline, C1D15, Surgery	Yes	-80ºC
Germline DNA for research	Whole blood	EDTA (purple)	10mL	Baseline	No	Ambient
Whole blood for plasma circulating DNA	Whole blood	Cell-free DNA BCT (Streck)	10mL x2	Baseline, C1D15, Surgery, Yearly post-surgery for 5 years	No	Room temperature, same day or overnight shipment (avoid Fridays)
Blood for detection of circulating tumor cells	Whole blood	EDTA (purple)	10 mL	Baseline	No	Dr. Aft's lab

9.2.2 Handling of Specimen(s)

9.2.2.1 Biopsies

For biopsies at each time point: 4 cores (14 G core needle) will be taken at each time point. Care needs to be taken to reduce the ischemic time to as much as possible to less than 30 min.

Samples can be obtained at the time of surgery. Care needs to be taken to reduce the ischemic time to as much as possible to less than 30 minutes.

An additional 20 of 5 micron section unstained slides from a tumor rich block of the resected breast cancer following surgery are requested. Alternatively, a tumor block could be submitted, which will be returned after sectioning. These sections or the tumor block with the completed Archival Tumor Specimen Submission Form (Appendix G) are to be shipped to Dr. Cynthia Ma's laboratory at the address in Section 9.1.

Additional tumor samples from surgical pathology may be requested if the research core does not contain sufficient tumor for correlative studies.

Secondary sites will not be submitting PDX samples.

Tissue will be distributed as follows:

- Baseline: 1 core fresh for PDX, 1 core snap frozen to the Tissue Procurement Core (TPC), and 2 cores preserved in OCT to the TPC
- C1D15: 1 core snap frozen to TPC, 1 core preserved in 10% formalin to TPC, and 2 cores preserved in OCT to TPC
- Surgery: 1 core fresh for PDX, 1 core snap frozen to TPC, and 2 cores preserved in OCT to TPC

9.2.2.2 Peripheral Blood

Serum and plasma processing: The blood samples must be processed to plasma and placed in the freezer at -80°C within one hour of collection. To process the blood samples to plasma, centrifuge the blood samples at approximately 4°C at 1700xg for approximately 10 minutes. Using a separate pipette for each time point, transfer the plasma samples into pre-labeled amber polypropylene cryovials and store at approximately -80°C until shipment. As much as practical, keep the blood and plasma samples away from direct sunlight and unfiltered lab light. Ship the samples on dry ice to the analytical labs.

Germ line DNA processing: The EDTA tube should be mixed several times and labeled with the patient's study number, date of birth, and collection date and time. Whole blood specimens are shipped in the specimen kit and must be received by the Washington University Tissue Procurement Facility within 48 hours of the time of collection. Do not freeze whole blood.

Whole blood for plasma circulating DNA: The cell free DNA collection BCT tube (provided with the kits) should be mixed several times and labeled with the patient's study number, date of birth, and collection date and time. Whole blood specimens are shipped in the specimen kit and must be received by the Washington University Tissue Procurement Facility

within 48 hours of the time of collection. Do not freeze whole blood. Do not put BCT tubes on ice.

9.2.3 Shipping of Specimen(s)

All samples should be labeled with institutional surgical pathology number (tumor samples), study number, patient ID number, patient initials, sample collection date and time and be accompanied by the completed specimen submission forms.

All samples should be shipped to the Wash U Tissue Procurement Core Facility.

Specimens may be sent to the Wash U Tissue Procurement Facility on Monday through Thursday for next day delivery. The Bank cannot receive specimens on Saturdays, Sundays, or holidays. Do not send specimens on Friday, Saturday, or the day before a holiday.

The institution is expected to pay the cost of mailing specimens and will be reimbursed through capitation fees set for each individual study.

Arrange for Federal Express pick-up through your usual institutional procedure. Ship specimens to the address below:

Wash U Tissue Procurement Core
425 S. Euclid Ave, Room 5120
St. Louis, MO 63110-1005
Phone: (314) 454-7615
E-mail: tbank@wudosis.wustl.edu

On the day that specimens are sent to the specimen bank, please contact the bank by phone, fax, or e-mail to notify what is being sent and when the shipment is expected to arrive.

9.3 OPTIONAL Bone Marrow Biopsy

9.3.1 Collection of Specimen(s)

Bone marrow aspirations for DTCs will be obtained only from patients who consent at the following two time points:

- Baseline
- At definitive surgery

Bone marrow collection and analysis will be paid for by research funds and will not be charged to the patient or patient's insurance.

The baseline bone marrow aspiration will be performed in the operating room at the time of port placement if possible to avoid a second procedure. The 2nd bone marrow aspiration will be performed in the operating room at the time of definitive

surgery.

Sixteen mL of bone marrow aspirate will be collected from the right and/or left iliac crest. After the collection of 8 mL the needle will be redirected to minimize the collection of peripheral blood. If the patient tolerates the collection of bone marrow from one side, then bone marrow will be collected from the contralateral side if needed to obtain additional aspirate.

The right or left posterior superior iliac crest region or the anterior iliac crest will be identified, and the area will be sterilely prepped with an alcohol based solution.

The region to be entered will be anesthetized with lidocaine and/or lidocaine/bupivacaine mixture and, following adequate anesthesia, an Illinois or Jamshidi needle will be inserted into the iliac crest region.

The bone marrow cavity will be penetrated and approximately 16 mL of bone marrow aspirate will be obtained from the iliac crest. If BM is collected from both iliac crests, then 1-4 mL of BM from each side will be combined and placed in a tube provided by a commercial vendor for DTC enumeration/Her2+ determination, 1-4 ml will be taken to a research lab for enrichment by filtration and molecular analysis. The remainder of the BM will be divided between 1-3 EDTA tubes. The procedure will be repeated on the contralateral side. After aspiration, the needle will be removed. The puncture sites will be cleaned and bandaged. Each participant will receive printed wound care instructions containing contact information for medical assistance if the participant experiences any adverse symptoms or problems following the procedure.

9.3.2 Handling of Specimen(s)

All tubes containing bone marrow will be labeled with UPN and the site of collection.

One to 2 EDTA tubes will be taken to a research laboratory for DTC enrichment and molecular analysis. If analyzed, two of the tubes will be used for RT-PCR analysis, and one tube will be stored. The Cee Sure tubes or tubes from other commercial vendors will be shipped in the kits provided by the vendor to Biocept or other commercial vendor according to the vendor's instructions. The EDTA tubes will be transported to the Siteman Cancer Center Tissue Procurement Core for processing and storage.

All tubes from non-WU sites will be shipped at room temperature to the WU Tissue Procurement Core (see Section 9.1.3).

10.0 STUDY CALENDAR

Screening/baseline evaluations are to be conducted within 4 weeks prior to start of protocol therapy. Scans and x-rays must be done no more than 12 weeks prior to the start of the protocol therapy. There is a +/- 1 day window for each study visit.

	Screening	**Baseline**	**Day 1 of Each Cycle[5]**	**C1D15**	**End of C1**	**End of C4**	**Pre-Surgery**	**Surgery**	**Post-Surgery[11]**	**Follow-Up[1]**
Informed consent	X									
H&P	X		X			X			X	
Vital signs	X		X			X			X	
CBC w/diff	X		X	X[10]		X			X	
CMP	X		X			X			X	
Pregnancy test[2]	X									
12-lead ECG	X									
Echocardiogram or MUGA[12]	X									
Mammogram	X[4]						X			
Ultrasound	X[4]						X			
Clinical evaluation			X							
Palbociclib			X[6]							
Trastuzumab (or FDA approved biosimilar)[13]			X[7]							
Letrozole			X[8]							
Goserelin[3]			X							
Research tumor tissue		X		X				X		
Research bone marrow[9]		X						X		
Research blood		X		X				X		X
Blood for germline DNA		X								
NCI PRO-CTCAE		X			X	X				
Adverse events assessment			Collected from prior to the first treatment to 30 days after the last dose of palbociclib							

1. Yearly post-surgery for 5 years for recurrence and survival
2. Women of childbearing potential only
3. Premenopausal women only

4. Must include bi-dimensional breast measurements, but retrospective measurement is allowed
5. Each cycle is 28 days; total of 4 cycles
6. Days 1-21 of each cycle, total of 4 cycles
7. Weekly (Days 1, 8, 15, and 22) of each cycle; after completion of 16 weeks, Trastuzumab maintenance will continue every 3 weeks
8. Daily until day of surgery
9. OPTIONAL
10. Also D15 of C2
11. Visit should coincide with Trastuzumab administration
12. After baseline, perform as per standard of care.
13. Trastuzumab or any of the FDA approved commercially available biosimilar products for trastuzumab may be administered to patients based on their insurance requirements or institutional practice.

11.0 DATA SUBMISSION SCHEDULE

Case report forms with appropriate source documentation will be completed according to the schedule listed in this section.

Case Report Form	Submission Schedule
On Study Form	Baseline
Treatment Form Clinical Measurements Form	End of each cycle
NCI PRO-CTCAE Form	Baseline End of Cycle 1 End of Cycle 4
Correlatives Form	Baseline Cycle 1 Day 15 Surgery Annually after surgery for 5 years
Imaging Form	Baseline Pre-Surgery
Surgery Form	Surgery
Treatment Summary Form	Off treatment
Follow-Up Form	Annually after surgery for 5 years
AE Form	Continuous
MedWatch	As described in Section 7.0

Any queries generated by Washington University must be responded to within 28 days of receipt by the participating site. The Washington University research team will conduct a regular review of data status at all secondary sites, with appropriate corrective action to be requested as needed.

11.1 Adverse Event Collection in the Case Report Forms

All adverse events that occur beginning with start of treatment (minus exceptions defined in Section 1.0) must be captured in the Toxicity Form. Baseline AEs should be captured on the Medical History Form.

Participant death due to disease progression should be reported on the Toxicity Form as grade 5 disease progression. If death is due to an AE (e.g. cardiac disorders: cardiac arrest), report as a grade 5 event under that AE. Participant death must also be recorded on the Death Form.

12.0 MEASUREMENT OF EFFECT

12.1 Neoadjuvant Treatment

Clinical Evaluation: Prior to Cycle 1, and at the beginning of each neoadjuvant treatment

cycle (that is, at the beginning of Cycles 2-4) the longest axis and the perpendicular axis of the measurable lesion should be measured and recorded in metric notation by tape, ruler or caliper technique on the case report forms. Clinical assessments will be done with bi-dimensional measurements using either tape, ruler, or calipers in centimeters. If discrete breast masses are not palpable, measurements will be recorded as 0 X 0 cm. The clinical measurements will be used to evaluate the best treatment response by clinical evaluation. The final response will be documented by a Principal Investigator Note to File and entered on the Treatment Summary Case Report Form.

Radiologic evaluation of tumor size: Mammogram and ultrasound imaging will be performed up to 12 weeks prior to Cycle 1 and at the end of Cycle 4 combination therapy for bidimensional measurement of the tumor. The baseline imaging is used for baseline measurement as study allows radiographic size or physical exam for eligibility. The pre-surgery imaging will be used to compare with baseline imaging to determine the % change from baseline.

WHO criteria will be used to assess clinical and radiologic responses.

Complete Response (CR) is defined as the disappearance of all known disease based on a comparison between the pre-treatment measurements and the measurements taken at the completion of neo-adjuvant therapy (that is, at the end of cycle 4 neo-adjuvant combination therapy). In addition, there is no appearance of new lesions.

Partial Response (PR) is defined as a 50% or greater decrease in the product of the bi-dimensional measurements of the lesion (total tumor size) between the pre-treatment measurements and the measurements taken at the completion of neo-adjuvant therapy (that is, at the end of cycle 4 neo-adjuvant combination therapy). In addition, there can be no appearance of new lesions or progression of any lesion.

No Change (NC): a 50% decrease in total tumor size cannot be established nor has a 25% increase in the size of the lesion been demonstrated.

Progressive Disease (PD): A 25% or greater increase in the total tumor size of the lesion from its pretreatment measurements or the appearance of new lesions.

12.2 Treatment Resistance

A patient is said to have resistance disease if clinical with confirmed radiographical progressive disease is documented any time during neoadjuvant endocrine therapy.

12.3 Surgery

A pathologic complete response (pCR) is defined as no histology evidence of invasive tumor cells in the surgical breast specimen and sentinel or axillary lymph nodes.

12.4 Post-surgery

Local recurrence is defined as histologic evidence of ductal carcinoma in situ or invasive breast cancer in the ipsilateral breast or chest wall.

Regional recurrence is defined as the cytologic or histologic evidence of disease in the ipsilateral internal mammary, ipsilateral supraclavicular, ipsilateral infraclavicular and/or ipsilateral axillary nodes or soft tissue of the ipsilateral axilla.

Distant recurrence is defined as the cytologic, histologic, and/or radiographic evidence of disease in the skin, subcutaneous tissue, lymph nodes (other than local or regional metastasis), lung, bone narrow, central nervous system or histologic and/or radiographic evidence of skeletal or liver metastasis.

Second primary breast cancer is defined histologic evidence of ductal carcinoma in situ or invasive breast cancer in the contralateral breast or chest wall.

Second primary cancer (non-breast) is defined as any non-breast second primary cancer other than squamous or basal cell carcinoma of the skin, melanoma in situ, or carcinoma in situ of the cervix is to be reported and should be confirmed histologically whenever possible.

13.0 DATA AND SAFETY MONITORING

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, an independent Data and Safety Monitoring Board (DSMB) will be specifically convened for this trial to review toxicity data at least every 6 months following the activation of the first secondary site. A DSMB will consist of no fewer than 3 members including 2 clinical investigators and a biostatistician. DSMB members must be employed by Washington University, Barnes-Jewish Hospital, or St. Louis Children's Hospital. Like investigators, DSMB members are subject to the Washington University School of Medicine policies regarding standards of conduct. Individuals invited to serve on the DSMB will disclose any potential conflicts of interest to the trial principal investigator and/or appropriate university officials, in accordance with institution policies. Potential conflicts that develop during a trial or a member's tenure on a DSMC must also be disclosed.

Until such a time as the first secondary site enrolls its first patient, a semi-annual DSM report to be prepared by the study team will be submitted to the Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after study activation at Washington University (if at least one patient has been enrolled) or one year after study activation (if no patients have been enrolled at the six-month mark).

The DSM report for the DSMB will be prepared by the study team with assistance from the study statistician, will be reviewed by the DSMB, and will be submitted to the Quality Assurance and Safety Monitoring Committee (QASMC). This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual including numbers from participating sites
- Protocol activation date at each participating site
- Average rate of accrual observed in year 1, year 2, and subsequent years at each participating site
- Expected accrual end date and accrual by site
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities at all participating sites
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

Further DSMC responsibilities are described in the DSMC charter.

The study principal investigator and coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines (please refer to Section 7.0).

Refer to the Washington University Quality Assurance and Data Safety Monitoring Committee Policies and Procedures for full details on the responsibilities of the DSMC.
This is located on the QASMC website at https://siteman.wustl.edu/research/clinical-research-resources/protocol-office-prmcqasmc/.

14.0 AUDITING

As coordinating center of this trial, Washington University (via the Quality Assurance and Safety Monitoring Committee (QASMC)) will monitor each participating site to ensure that all protocol requirements are being met; that applicable federal regulations are being followed; and that the best practices for patient safety and data collection are being followed per protocol. Participating sites will be asked to send copies of all audit materials, including source documentation. The audit notification will be sent to the Washington University Research Patient Coordinator, who will obtain the audit materials from the participating institution.

Notification of an upcoming audit will be sent to the research team one month ahead of the audit. Once accrual numbers are confirmed, and approximately 30 days prior to the audit, a list of the cases selected for review (up to 10 for each site) will be sent to the research team. However, if during the audit the need arises to review cases not initially selected, the research team will be asked to provide the additional charts within two working days.

Items to be evaluated include:

- Subject screening and enrollment
- Reporting of adverse events
- Maintenance of HIPAA compliance
- Completeness of regulatory documentation
- Acquisition of informed consent
- IRB documentation
- Issues of protocol adherence

Additional details regarding the auditing policies and procedures can be found at https://siteman.wustl.edu/wp-content/uploads/2015/10/QASMC-Policies-and-Procedures-03.31.2015.pdf

15.0 STATISTICAL CONSIDERATIONS

15.1 Sample Size Calculation

We assume a null hypothesis of 15% pCR rate in this setting. We further hypothesize that a pCR rate of 30% or higher warrants further investigation. A one stage single arm phase II design was adopted for sample size planning. 48 eligible patients will be enrolled to achieve 80% power at a 1-sided 0.05 significance level. If we observe 12 or more pCRs, the treatment regimen shows better efficacy and warrants further investigation.

It is estimated based on the accrual rate of 2 patients per month across 3 participating centers that the completion of enrollment will take 24 months.

15.2 Data Analysis Plan

All data will be evaluated as observed, and no imputation method for missing values will be used. Descriptive statistics will be used to summarize the trial results, i.e., statistics for continuous variables may include means, medians, ranges and appropriate measures of variability such as standard deviation and inter-quartile range. Qualitative variables will be summarized by counts and percentages. The uncertainty of estimates will be assessed by confidence intervals.

The primary endpoint, pCR rate, defined as the proportion of patients with pCR in breast and axilla, will be calculated with 95% confidence interval. SAEs will be summarized by descriptive statistics. For the markers of interest in the correlative studies, Wilcoxon ranks um test or two sample t-test as appropriate will be conducted to compare a quantitative

marker between responders and non-responders while Fisher's exact test will be used for comparison of qualitative markers such as subtype and mutation. All analyses and estimates will be conducted among all patients overall, as well as by subgroups of interest.

Safety analyses will be done separately for the full intention-to-treat population and also for subjects who receive any study treatment. All patients are evaluable for disease response unless they discontinue treatment due to treatment-related adverse events prior to completion of Cycle 1.

15.3 Early Stopping Rule

Early stopping of this trial will be based on unacceptable toxicity or futility. Approximately less than 15% of patients are expected to experience grade 3 and up non-hematological toxicity and that a non-hematological toxicity rate of 33% or more would definitely be unacceptable. Based on the sequential probability ratio test (SPRT) with 80% power and 0.05 significance level, the study will be halted if 4 of the first 6, or 5 of the first 10, or 6 of the first 15, or 7 of the first 19, or 8 of the first 23, or 9 of the first 28, or 10 of the first 32, or if the14th toxicity is observed before the last patient has completed the trial. Accrual will be stopped and the event will be reviewed by the Data Safety and Monitoring Board if a grade 5 toxicity is observed.

Once the first 20 evaluable patients are available in the study, we will start sequential futility monitoring for pCR. A Bayesian sequential monitoring method (Thall et al. 1995, 1996; Ivanova et al 2005) will be implemented for futility analysis using a free software "Multc Lean" (version 2.1.0) from M.D Anderson Cancer Center (https://biostatistics.mdanderson.org/softwaredownload/SingleSoftware.aspx?Software_Id=12). The stopping rule is defined as Pr ($\theta_E > \theta_0$ |observed data)<0.10, with θ_E denoting the pCR rate evaluated against a cutoff value θ_0 and observed data referring to evaluable data in the study at the time of evaluation. Since the literature showed a pCR rate of 15% with standard neoadjuvant treatment regimen in this patient population with letrozole and trastuzumab, we will set the cutoff θ_0=20% in the current monitoring plan. The above stopping rule is interpreted as: the study will stop when there is <10% probability that the combination treatment with letrozole, palbociclib, and trastuzumab achieves a pCR rate above 20%. The stopping boundaries on the number of pCRs where the posterior probability of the combination treatment regimen being less responsive (<0.2) is greater than 0.9 were calculated using "Multc Lean". Specifically, we assumed that θ_E follows a beta distribution, $\theta_E \sim$ beta ($\alpha + x$, $\beta + n - x$), where x and n-x represent the observed numbers of pCRs and non-pCRs respectively, while α and β is the shape and scale parameter for a prior beta distribution. We adopted the prior distribution beta (0.2, 0.48), which corresponds to a mean of 0.2 and a relatively large variance of 0.1, serving as a non-informative prior. The trial will be recommended to stop for futility if we observe at most 2 subjects with pCR out of the first 20-26 evaluable patients, at most 3 out of the first 28-32 patients, at most 4 out of the first 34-38 patients, at most 5 out of 40-44 patients and at most 6 out of 48 patients.

15.4 Accrual Strategy

a) Use our weekly breast cancer research meeting to identify potentially eligible patients for PALTAN that will be seen by our breast medical oncologists. There are currently **NO COMPETING** trials for this population.
b) Disseminate availability of the PALTAN trial to local medical and surgical community oncologists via letters.
c) Provide ongoing feedback to referring physicians while their patients are on PALTAN.
d) Involvement of patient advocate in education and in trial promotion. We have involved a breast cancer patient advocate, Judy Johnson, who has been involved in the design of this clinical protocol and consent form from a patient's perspective. Judy Johnson will also assist with the development of a patient brochure for PALTAN.
e) Identify and address reasons why eligible patients decline PALTAN trial participation, via screening logs to be reviewed every month by PI and study clinical research associate.
f) Engagement of minority recruitment specialist. We will maximize recruitment of minorities through assistance of Jessica Thein, who works at our cancer center to increase minority participation in clinical trials.
g) Employ the use of social media (FACEBOOK) to enhance recruitment to via posting a link to the PALTAN patient brochure on the Siteman Cancer Center Facebook page.
h) Provide access to peer mentors (other patients who have participated in other clinical trial) and patient navigators for those patients identified as in need of additional support.
i) Include multilingual staff and medical interpreters as members of the PALTAN research team as the need arises.

16.0 MULTICENTER REGULATORY REQUIREMENTS

Washington University requires that each participating site sends its informed consent document to be reviewed and approved by the Washington University Regulatory Coordinator (or designee) prior to IRB/IEC submission.

Site activation is defined as when the secondary site has received official written documentation from the coordinating center that the site has been approved to begin enrollment. At a minimum, each participating institution must have the following documents on file at Washington University prior to study activation:

- Documentation of IRB approval of the study in the form of a letter or other official document from the participating institution's IRB. This documentation must show which version of the protocol was approved by the IRB.
- Documentation of IRB approval of an informed consent form. The consent must include a statement that data will be shared with Washington University, including the Quality

Assurance and Safety Monitoring Committee (QASMC), the DSMC (if applicable), and the Washington University study team.
- Documentation of FWA, signed FDA Form 1572 (if applicable), and the CVs of all participating investigators.
- Protocol signature page signed and dated by the investigator at each participating site.

The coordinating center Principal Investigator (or designee) is responsible for disseminating to the participating sites all study updates, amendments, reportable adverse events, etc. Protocol/consent modifications and IB updates will be forwarded electronically to the secondary sites within 4 weeks of obtaining Washington University IRB approval. Activated secondary sites are expected to submit protocol/consent/IB modifications to their local IRBs within 4 weeks of receipt unless otherwise noted. Upon the secondary sites obtaining local IRB approval, documentation of such shall be sent to the Washington University study team within 2 weeks of receipt of approval.

Documentation of participating sites' IRB approval of annual continuing reviews, protocol amendments or revisions, all SAE reports, and all protocol violations/deviations/exceptions must be kept on file at Washington University.

The investigator or a designee from each institution must participate in a regular conference call to update and inform regarding the progress of the trial.

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We would like to thank the Alvin J. Siteman Cancer Center at Washington University School of Medicine and Barnes-Jewish Hospital in St. Louis, Missouri, for the use of the Clinical Trials Core which provided protocol development services. The Siteman Cancer Center is supported in part by an NCI Cancer Center Support Grant #P30 CA91842.

APPENDIX A: ECOG Performance Status Scale

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

APPENDIX B: Strong CYP3A4 Inhibitors or Inducers
(http://medicine.iupui.edu/clinpharm/ddis/clinical-table/)

Inhibitors
Indinavir
Nelfinavir
Ritonavir
Clarithromycin
Itraconazole
Ketoconazole
Nefazodone

Inducers
Carbamazepine
Efavirenz
Nevirapine
Phenobarbital
Phenytoin
Pioglitazone
Rifabutin
Rifampin
St. John's Wort
Troglitazone

APPENDIX C: Pfizer Reportable Event Cover Sheet

Investigator-Initiated Research
Reportable Event Fax Cover Sheet

Use this fax cover sheet to fax a Reportable Event for Investigator-Initiated Research studies.

Include with this form the completed Pfizer Investigator-Initiated Research Serious Adverse Event (IIR SAE) form, MedWatch Form FDA 3500A-Mandatory Reporting, which can be obtained from the FDA website: www.fda.gov/medwatch/getforms.htm, or other Pfizer agreed-upon form for SAE reporting.

If you are using the MedWatch Form to report, the following information should be included in block 5 of the Adverse Events section:

- **The complete clinical course of the patient receiving Pfizer drug**
- **The causality assessment for each Reportable Event**
- **The action taken for each study drug and for each Reportable Event**
- **The outcome for each Reportable Event**

This cover sheet MUST be provided with each completed SAE form. Do not substitute forms/reports or submit additional documentation other than what is required.

Do not fax these forms to any additional fax numbers other than the one listed below.

TO:	*Pfizer U.S. Clinical Trial Department*		
FAX:	*1-866-997-8322*		
FROM:		DATE:	
TELEPHONE:		FAX:	
NUMBER OF PAGES (INCLUDING COVER SHEET):			
PRODUCT	PRODUCT NAME		
PFIZER REFERENCE NUMBER	TRACKING NUMBER	EXTERNAL REFERENCE NUMBER	EXTERNAL REFERENCE
STUDY TITLE	*STUDY TITLE*		
PATIENT NUMBER			
INVESTIGATOR	INVESTIGATOR NAME, DEGREE		

FormCT26-USA01-10 Reportable Event Fax Cover Sheet: US_eff 19-DEC-2006

APPENDIX D: PALBOCICLIB MEDICATION DIARY

Today's Date: _____ Agent: Palbociclib

Cycle: _____ Study ID#: _____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each month. Take _____mg (___capsules) of palbociclib at approximately the same time each day with food. Swallow the capsules whole and do not chew them.
2. Record the date, the number of capsules taken, and when you took them.
3. If you forgot to take your dose before 6:00PM, then do not take a dose that day. Restart taking it the next day.
4. If you have any questions or notice any side effects, please record them in the comments section. Record the time if you should vomit.
5. Please return the forms to your physician or your study coordinator when you go to your next appointment. Please bring your unused study medications and/or empty bottles with you to each clinic visit so that a pill count can be done.
6. Avoid St. John's Wort, Seville oranges, grapefruit, grapefruit juice, grapefruit hybrids, pummelos, and exotic citrus fruits from 7 days before you start taking palbociclib and throughout the entire study.

Day	Date	What time was dose taken?	# of capsules taken	Comments
1				
2				
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4				
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6				
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APPENDIX E: LETROZOLE MEDICATION DIARY

Today's Date: _____ Agent: Letrozole

Cycle: _____ Study ID#: _____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each month. Take _____mg (___tablets) of letrozole at approximately the same time each day with or without food. Swallow the tablets whole and do not chew them.
2. Record the date, the number of tablets taken, and when you took them.
3. If you forgot to take your dose before 6:00PM, then do not take a dose that day. Restart taking it the next day.
4. If you have any questions or notice any side effects, please record them in the comments section. Record the time if you should vomit.
5. Please return the forms to your physician or your study coordinator when you go to your next appointment. Please bring your unused study medications and/or empty bottles with you to each clinic visit so that a pill count can be done.

Day	Date	What time was dose taken?	# of tablets taken	Comments
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APPENDIX F: NCI PRO-CTCAE QUESTIONNAIRE

NCI PRO-CTCAE™ ITEMS

Item Library Version 1.0

As individuals go through treatment for their cancer they sometimes experience different symptoms and side effects. For each question, please check or mark an ☒ in the one box that best describes your experiences over the past 7 days...

1.	In the last 7 days, what was the SEVERITY of your DECREASED APPETITE at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe
	In the last 7 days, how much did DECREASED APPETITE INTERFERE with your usual or daily activities?				
	○ Not at all	○ A little bit	○ Somewhat	○ Quite a bit	○ Very much

2.	In the last 7 days, how OFTEN did you have NAUSEA?				
	○ Never	○ Rarely	○ Occasionally	○ Frequently	○ Almost constantly
	In the last 7 days, what was the SEVERITY of your NAUSEA at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe

3.	In the last 7 days, what was the SEVERITY of your SHORTNESS OF BREATH at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe
	In the last 7 days, how much did your SHORTNESS OF BREATH INTERFERE with your usual or daily activities?				
	○ Not at all	○ A little bit	○ Somewhat	○ Quite a bit	○ Very much

4.	In the last 7 days, what was the SEVERITY of your COUGH at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe
	In the last 7 days, how much did COUGH INTERFERE with your usual or daily activities?				
	○ Not at all	○ A little bit	○ Somewhat	○ Quite a bit	○ Very much

5.	In the last 7 days, how OFTEN did you have ARM OR LEG SWELLING?				
	○ Never	○ Rarely	○ Occasionally	○ Frequently	○ Almost constantly
	In the last 7 days, what was the SEVERITY of your ARM OR LEG SWELLING at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe
	In the last 7 days, how much did ARM OR LEG SWELLING INTERFERE with your usual or daily activities?				
	○ Not at all	○ A little bit	○ Somewhat	○ Quite a bit	○ Very much

6.	In the last 7 days, how OFTEN did you feel a POUNDING OR RACING HEARTBEAT (PALPITATIONS)?				
	○ Never	○ Rarely	○ Occasionally	○ Frequently	○ Almost constantly
	In the last 7 days, what was the SEVERITY of your POUNDING OR RACING HEARTBEAT (PALPITATIONS) at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe

7.	In the last 7 days, did you have any RASH?	
	○ Yes	○ No

8.	In the last 7 days, what was the SEVERITY of your DRY SKIN at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe

9.	In the last 7 days, did you have any HAIR LOSS?				
	○ Not at all	○ A little bit	○ Somewhat	○ Quite a bit	○ Very much

10.	In the last 7 days, what was the SEVERITY of your PROBLEMS WITH CONCENTRATION at their WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe
	In the last 7 days, how much did PROBLEMS WITH CONCENTRATION INTERFERE with your usual or daily activities?				
	○ Not at all	○ A little bit	○ Somewhat	○ Quite a bit	○ Very much

APPENDIX G: ARCHIVAL TUMOR SPECIMEN SUBMISSION FORM

Laboratory of Dr. Cynthia X. Ma
Archival Tumor Specimen Submission Form

HRPO ID#: 201610019 **Submitter Last Name:** _____

Participant Study #: _____ **Submitter First Name:** _____

Participant Name (Initials): Last: First: Middle: ____ **Submitter's Phone #:** _____

Collection Site (*select one*): ☐Washington University School of Med ☐Roswell Park ☐ Mayo Arizona

Study Time Point: ARCHIVE

Specimen(s) Submitted: *Clinical Research Coordinator to provide date & time collected and # of specimens. Include completed form with specimen shipment.*

Parent Label	Parent Type	Date & Time Collected	Number of Specimens	Site of Tissue
	☐Fixed tissue block ☐Fixed tissue slide			☐diagnostic core biopsy of the primary breast cancer
	☐Fixed tissue block ☐Fixed tissue slide			☐surgical resection of the breast cancer post neoadjuvant therapy

Processing Notes:
10 of 5 micron section unstained slides from the pretreatment archival diagnostic core biopsy of the primary breast cancer as well as 20 of 5 micron unstained slides from a tumor rich block of the resected breast cancer following neoadjuvant therapy are requested. Alternatively, a tumor block could be submitted, which will be returned after sectioning. Additional tissue may be requested if the samples are insufficient. Please include pathology report.

Shipment Address:
Dr. Cynthia Ma Laboratory
Attn: Jeremy Hoog
Washington University School of Medicine
4515 McKinley Research Building
Campus Box 8076
3rd Floor, Room 3111A
St. Louis, MO 63110
Phone: (314) 747-9309

APPENDIX H: Definitions for Adverse Event Reporting

A. Adverse Events (AEs)

As defined in 21 CFR 312.32:

Definition: any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug-related.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website:
http://www.hhs.gov/ohrp/policy/advevntguid.html

B. Suspected Adverse Reaction (SAR)

As defined in 21 CFR 312.32:

Definition: any adverse event for which there is a reasonable possibility that the drug caused the adverse event. "Reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. "Suspected adverse reaction" implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

C. Life-Threatening Adverse Event / Life Threatening Suspected Adverse Reaction

As defined in 21 CFR 312.32:

Definition: any adverse drug event or suspected adverse reaction is considered "life-threatening" if, in the view of the investigator, its occurrence places the patient at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

D. Serious Adverse Event (SAE) or Serious Suspected Adverse Reaction

As defined in 21 CFR 312.32:

Definition: an adverse event or suspected adverse reaction is considered "serious" if, in the view of the investigator, it results in any of the following outcomes:

- Death
- A life-threatening adverse event

- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Any other important medical event that does not fit the criteria above but, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

E. Protocol Exceptions

Definition: A planned change in the conduct of the research for one participant.

F. Deviation

Definition: Any alteration or modification to the IRB-approved research without prospective IRB approval. The term "research" encompasses all IRB-approved materials and documents including the detailed protocol, IRB application, consent form, recruitment materials, questionnaires/data collection forms, and any other information relating to the research study.

A minor or administrative deviation is one that does not have the potential to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study.

A major deviation is one that does have the potential to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study.

APPENDIX I: Reporting Timelines

Expedited Reporting Timelines					
Event	**HRPO**	**QASMC**	**FDA**	**IBC**	**Pfizer**
Serious AND unexpected suspected adverse reaction			Report no later than 15 calendar days after it is determined that the information qualifies for reporting	N/A	Within 24 hours of first awareness of the event
Unexpected fatal or life-threatening suspected adverse reaction			Report no later than 7 calendar days after initial receipt of the information		Within 24 hours of first awareness of the event (immediately if the event is fatal or life-threatening)
Unanticipated problem involving risk to participants or others	Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day.	Report via email after IRB acknowledgment			
Major deviation	Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day.				
A series of minor deviations that are being reported as a continuing noncompliance	Report within 10 working days.				
Protocol exception	Approval must be obtained prior to implementing the change				
Clinically important increase in the rate of a serious suspected adverse reaction of			Report no later than 15 calendar days after it is determined that the		

Expedited Reporting Timelines					
Event	**HRPO**	**QASMC**	**FDA**	**IBC**	**Pfizer**
that list in the protocol or IB			information qualifies for reporting		
Complaints	If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance, report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review.				
Breach of confidentiality	Within 10 working days.				
Incarceration	If withdrawing the participant poses a safety issue, report within 10 working days. If withdrawing the participant does not represent a safety issue and the patient will be withdrawn, report at continuing review.				

Routine Reporting Timelines				
Event	**HRPO**	**QASMC**	**FDA**	**Pfizer**
Adverse event or SAE that does not require expedited reporting	If they do not meet the definition of an unanticipated problem involving risks to participants or others, report summary information at the time of continuing review	Adverse events will be reported in the toxicity table in the DSM report which is typically due every 6 months.	The most current toxicity table from the DSM report is provided to the FDA with the IND's annual report.	
Minor deviation	Report summary information at the time of continuing review.			
Complaints	If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance, report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review.			
Incarceration	If withdrawing the participant poses a safety issue, report within 10 working days. If withdrawing the participant does not represent a safety issue and the patient will be withdrawn, report at continuing review.			
Pregnancy and/or lactation				Within 24 hours of first awareness of the event (immediately if the event is fatal or life-threatening)
Occupation Exposure				Within 24 hours of first awareness of the event (immediately if the event is fatal or life-threatening)
Hy's Law Case (Drug-Induced Liver Injury)				Within 24 hours of first awareness of the event

				(immediately if the event is fatal or life-threatening)

Expedited Reporting Timelines for Secondary Sites				
Event	**WU (Coordinating Center)**	**Local IRB**	**FDA**	**Pfizer**
Serious AND unexpected suspected adverse reaction	Report no later than 11 calendar days after it is determined that the information qualifies for reporting.	Report all applicable events to local IRB according to local institutional guidelines.	The research team at Washington University is responsible for reporting all applicable events to the FDA as needed.	The research team at Washington University is responsible for reporting all applicable events to Pfizer as needed.
Unexpected fatal or life-threatening suspected adverse reaction	Report no later than 4 calendar days after initial receipt of the information.			
Unanticipated problem involving risk to participants or others	Report no later than 4 calendar days after initial receipt of the information.			
Adverse event or SAE that does not require expedited reporting	As per routine data entry expectations			
Protocol exception	Approval must be obtained prior to implementing the change.			

APPENDIX J: Washington University SAE Reporting Cover Sheet

SAE COVER SHEET- Secondary Site Assessment

Washington University HRPO#:	Sponsor-Investigator:
Subject Initials:	Subject ID:
Treating MD:	Treating Site:
EVENT TERM:	Event Start Date:
EVENT GRADE:	Date of site's first notification:

Treating MD Event Assessment:

Is this event **possibly, probably, or definitely** related study treatment?

☐ yes ☐ no

If yes, please list which drug (if more than one)_____

Explain _____

_____

_____ _____ _____

Physician's Name **Physician's Signature** **Date**