A phase II clinical trial assessing the safety of an alternative dosing schedule of palbociclib in metastatic hormone receptor positive breast cancer

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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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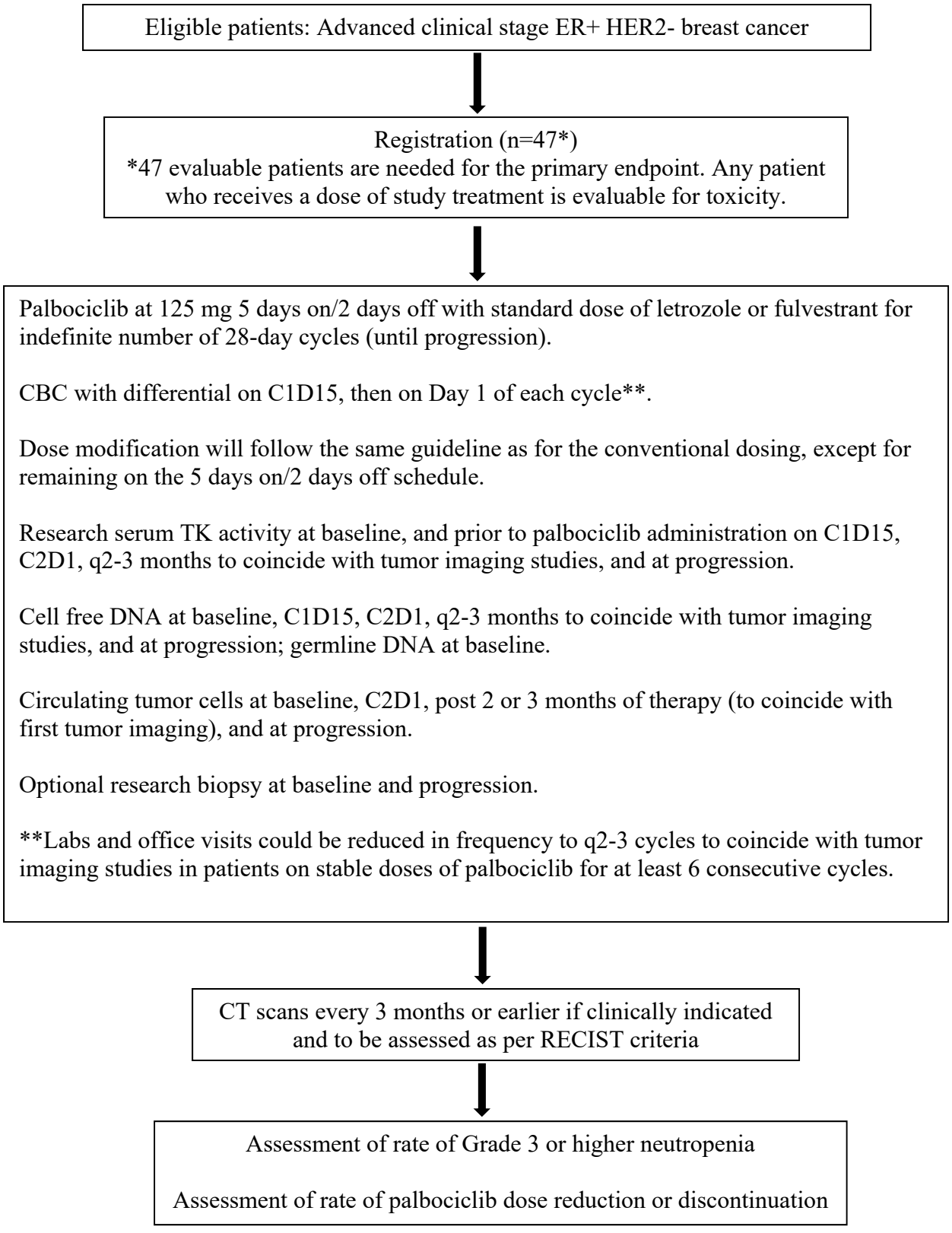

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1.0 BACKGROUND AND RATIONALE

1.1 Hormone Receptor Positive Breast Cancer

Hormone receptor positive (estrogen receptor (ER+) and/or progesterone receptor (PR+)) breast cancers account for nearly 80% of breast cancer diagnoses. Endocrine therapies are the cornerstone of treatment for these cancers. A meta-analysis by the Early Breast Cancer Trialists' Collaborative Group showed that these agents substantially reduce the relapse rate and mortality in the adjuvant setting for early stage breast cancers that were ER+ or PR+ (1).

In spite of advances in endocrine therapy, a considerable clinical challenge remains in the form of many women presenting with systemic relapse of breast cancer either during or after completion of adjuvant hormone therapy. In aromatase inhibitor (AI) resistant setting, hormonal therapy such as a different AI, tamoxifen or the selective estrogen-receptor down regulator fulvestrant, as single agents have shown limited clinical benefit (2-4). Although the mTOR inhibitor everolimus has shown to improve PFS in the AI resistant setting, its activity is modest and is associated with significant toxicity including mucositis and hyperglycemia (5).

Cyclin D1–CDK4–CDK6 (CDK- Cyclin dependent kinase) complex is a key downstream effector in ER+ and PR+ breast cancer and retains its function even after development of resistance to endocrine therapy (6,7). Targeting CDK4 and CDK6 is an attractive strategy to overcome this resistance.

1.2 Palbociclib (Ibrance)

Palbociclib (Ibrance, Pfizer) is a highly selective small-molecule inhibitor of CDK4 and CDK6, which can be administered orally. It results in loss of RB1 phosphorylation in vitro and leads to cell cycle arrest at the G1-S phase in ER+ breast cancer (8). While initial phase 2 studies demonstrated single agent activity in hormone receptor positive breast cancer (9), subsequent studies have reported synergistic activity with endocrine therapies in ER+/PR+ breast cancer. In preclinical studies, palbociclib was effective in both tamoxifen sensitive and resistant ER+ breast cancer cell lines (7).

An open-label randomized phase 2 study involving postmenopausal patients with newly diagnosed metastatic ER+ and human epidermal growth factor receptor (Her-2/neu) negative breast cancer (PALOMA1), showed that palbociclib in combination with letrozole was associated with significantly prolonged PFS when compared with letrozole alone (10). This resulted in an accelerated FDA approval of palbociclib for use in this specific patient population (11). In this trial, 27 patients in the palbociclib plus letrozole group (33%) had dose interruptions because of adverse events, compared with only three (4%) patients in the letrozole group. In the combination group, 37 (45%) patients required a delay in the start of a subsequent treatment cycle because of an adverse event and 33 (40%) patients had a dose reduction. In this study, 11 (13%) patients in the palbociclib plus letrozole group

and two (2%) patients in the letrozole group discontinued the study because of an adverse event. Of these discontinuations, six (7%) patients in palbociclib plus letrozole group and two (2%) patients in the letrozole group discontinued because of treatment-related adverse events.

A randomized phase 3 study to confirm the efficacy of palbociclib in combination with letrozole in this setting (PALOMA2) was recently reported at ASCO 2016 annual meeting and again demonstrated a significant improvement in progression free survival (PFS): 24.8 (22.1-NR) months in the palbociclib/letrozole arm vs 14.5 (12.9-17.1) months in the placebo/letrozole arm, HR 0.58; $p<0.000001$ (12). Among the 444 patients treated with palbociclib and letrozole, neutropenia (any grade) occurred in 80% of patients vs. 6% in the placebo/letrozole arm. The incidence of grade 3 and 4 neutropenia in the palbociclib/letrozole arm was 56% and 10%, respectively. Grade 3 or 4 non-hematological AEs were uncommon. 9.7% of patients in the palbociclib/letrozole arm vs 5.9% in the placebo/letrozole arm discontinued treatment due to AEs (12).

Most recently, a randomized phase 3 study (PALOMA3) assessed the safety and efficacy of the combination of palbociclib and fulvestrant vs placebo and fulvestrant after disease progression after prior endocrine therapy in premenopausal and postmenopausal women with hormone receptor positive and Her-2/neu negative advanced breast cancer. In this study, premenopausal females also received goserelin for ovarian suppression. A significantly prolonged PFS was noted with the combination of palbociclib and fulvestrant compared with fulvestrant and placebo. The dosing schedule of palbociclib was 125 mg po daily for 3 weeks followed by one week off drug (13).

Similar to PALOMA2, most common hematologic adverse events reported for the palbociclib–fulvestrant group in PALOMA3 were neutropenia and leukopenia. Neutropenia (any grade) occurred in 78.8% of the patients receiving palbociclib–fulvestrant versus 3.5% in the placebo–fulvestrant group, leukopenia in 45.5% versus 4.1%, anemia in 26.1% versus 9.9%, and thrombocytopenia in 19.4% versus 0%. Grade 3 or 4 neutropenia occurred in 62.0% of the patients receiving palbociclib–fulvestrant versus 0.6% of the patients receiving placebo–fulvestrant, leukopenia in 25.2% versus 0.6%, anemia in 2.6% versus 1.7%, and thrombocytopenia in 2.3% versus 0%. The most common nonhematologic adverse events were fatigue, nausea (29.0% vs. 26.2%), and headache, all of which were more common in the palbociclib-fulvestrant group. A higher incidence of infections was also reported in the palbociclib–fulvestrant group (13).

Palbociclib dose was reduced in 109 of 345 patients (31.6%), whereas the placebo dose was reduced in 3 of 172 patients (1.7%). Discontinuation of palbociclib or matching placebo owing to adverse events occurred in 9 patients (2.6%) receiving palbociclib and 3 patients (1.7%) receiving placebo (13).

1.3 Study Rationale

The current dose modification guidelines for palbociclib are as follows:

CTCAE* grade	Dose modifications
Grade 1 or 2	No adjustment required
Grade 3	No dose adjustment is required. Consider repeating complete blood count monitoring one week later. Withhold initiation of next cycle until recovery to Grade ≤2
Grade 3 ANC (<1000 to 500/mm^3) + Fever ≥ 38.5C and/or infection	Withhold Palbociclib and initiation of next cycle until recovery to Grade ≤2 (≥1000/mm^3). Resume at next lower dose
Grade 4	Withhold Palbociclib and initiation of next cycle until recovery to Grade ≤2. Resume at next lower dose

*Common terminology criteria for adverse events

We propose to conduct a study to test an alternative dosing schedule of palbociclib. With the current three-week on and one week off schedule, a significant number of patients develop grade 3 or higher degree of neutropenia and require dose reduction and sometimes discontinuation. This potentially compromises the efficacy of the drug. In addition, as the half-life of palbociclib is 27 hours, 1 week break with the standard 3 weeks on and 1 week off dosing schedule could potentially lead to recovery of Rb phosphorylation during the off week. Hence, we propose a 5 days on and 2 days off schedule each week without any weeks off drug. Although the cumulative doses each 28-day cycle is roughly the same with this schedule compared to conventional dosing, the bone marrow is not exposed to the drug continuously for 21 days and rather gets frequent breaks from therapy. We hypothesize that the 5 days on and 2 days off schedule is more tolerable with less frequent high grade neutropenia and dose interruption/reduction. In addition, this schedule also provides for a more continuous drug delivery to the patient since there is not a week's break in therapy, which could ultimately prove to be more efficacious.

1.4 Correlative Studies Background

Serum TK1 activity

Thymidine kinase-1 (TK1) is an enzyme which plays a critical role in the synthesis of DNA. All human cells express this enzyme during normal cell division through E2F-dependent transcription with only small amounts of the enzyme released into the serum (14,15). Due to higher rates of replication compared to normal tissues, tumors are capable of secreting pathologic levels of TK1 which can be detected via an ELISA assay. In patients with metastatic breast cancer, TK1 expression and activity in human serum, was predictive for therapy response and correlated with PFS and OS (16). Given that TK1 expression is E2F-dependent, we hypothesize that CDK4 inhibitors, if active in a particular tumor, would result in reduced serum TK1 activity and can be used as a potential biomarker

for the pharmacodynamics effect of CDK4/6 inhibition. We will also evaluate changes in serum TK1 activity in relation to tumor response as an exploratory endpoint in this study.

Cell-free circulating tumor DNA (ctDNA) sequencing

Plasma samples from cancer patients often carry small amounts of fragmented cell-free DNA of 160-180 base pairs, which are originated from the necrosis or apoptotic process of cancer cells. Advances in the next generation sequencing (NGS) technology and digital genomic techniques support the clinical validity of cell-free circulating DNA (ctDNA) sequencing analysis to non-invasively identify actionable genomic alterations, monitor treatment response, and investigate resistance mechanisms (17). To investigate whether ctDNA sequencing could serve as an early predictor of response and provide clues of resistance mechanisms, blood for cell free tumor DNA sequencing will be collected before, during and at the time of progression to assess changes in the mutation profile including *PIK3CA, RB1, ESR1, TP53* and others. These genes are chosen as *PIK3CA, TP53* and *ESR1* are among the most common mutations in AI resistant ER+ breast cancer (18,19) and palbociclib has shown to be effective in ER pos breast cancers that carry these mutations (20,21). However, there is limited data on whether these mutations could be used to monitor treatment response and whether mutations in these genes could be acquired or enriched during or at progression on palbociclib. It is of particular importance whether *ESR1* mutation could develop at the time of acquired treatment resistance. The results may have implications on the development of subsequent treatment strategies. RB1 mutation has been implicated as a de novo and acquired treatment resistance mechanism to CDK4/6 inhibition (21-23), however, the incidence of RB1 mutation after progression on palbociclib combination regimen is unknown. This trial will provide this valuable data for future studies of targeted agents aimed to treat RB1 mutant tumors.

Tumor analysis

In addition to archival tumor specimens, in consented patients who have accessible tumors, tumor biopsies will be obtained before and/or after disease progression to examine mechanisms of intrinsic and acquired resistance mechanisms, by proteomic analysis including reverse phase protein array, immunohistochemistry of pRB, RNA expression profiling and targeted or whole exome sequencing studies depending on funding availabilities.

Patient derived xenograft (PDX)

PDX models allow the opportunity for in-depth molecular characterization of the tumor and investigation of mechanisms of response/resistance to treatments, therefore whenever possible, we will try to engraft biopsy samples to immunocompromised mice from consented patients as described in our previous publications (24).

Circulating tumor cells (CTC)

CTCs are rare events in the bloodstream, but may provide an accessible source of material for detection, characterization and monitoring of non-hematologic malignancies. Current estimates suggest that these cells are present in numbers as low as 1 to 10 in 10^9 blood cells. The majority of studies, including those in the setting of metastatic breast cancer, utilized the FDA-cleared CellSearch System for CTC enumeration, and showed the

prognostic value of CTC count in both progression free survival and overall survival (25). With the advances of technologies, we and others have developed new methods of CTC isolation that may allow for more accurate enumeration and even biochemical characterization of these cells (26,27). Recent studies have also demonstrated success in genomic, transcriptomic, and proteomic analyses in breast cancer CTCs (28,29). These data support the use of CTCs as valuable information source to non-invasively identify actionable genomic alterations, monitor treatment response, and investigate resistance mechanisms. To investigate whether CTC-based gene expression profiling could serve as an early predictor of response and provide clues of resistance mechanisms, blood will be collected before, during and at the time of progression to assess changes in the gene expression profile, including PAM50 molecular classifiers (30-32) and cell cycle genes and regulators. Very limited data exist regarding the molecular markers for predicting or monitoring treatment response and resistance to palbociclib, it has been reported that CDK4/6 inhibitor treatment shifted ER+/HER2- models from a high risk (luminal B) to a low risk (luminal A) molecular-phenotype (33). We will associate the baseline and shift of CTC-based molecular subtype with patients' clinical response. This trial will provide this valuable data for the development of non-invasive companion diagnostics or monitoring tools for patients to receive palbociclib.

2.0 OBJECTIVES

2.1 Primary Objective

To determine the rate of grade 3 or higher neutropenia in patients with hormone receptor positive breast cancer treated with palbociclib on a 5 days on/2 days off schedule within the first 29 days of treatment.

2.2 Secondary Objectives

1. To determine the rate of grade 3 or higher neutropenia in patients with hormone receptor positive breast cancer treated with palbociclib on a 5 days on/2 days off schedule during all cycles.
2. To determine the rate of palbociclib dose reduction, interruption, or discontinuation.
3. To determine the adverse events profile of palbociclib given on a 5 days on/2 days off schedule.
4. To determine the progression free survival in patients with hormone receptor positive breast cancer treated with palbociclib on a 5 days on/2 days off schedule.
5. To determine the overall response rate (CR+PR) in patients with hormone receptor positive breast cancer treated with palbociclib on a 5 days on/2 days off schedule.
6. To determine the clinical benefit rate (CR+PR+SD for at least 6 months) in patients with hormone receptor positive breast cancer treated with palbociclib on a 5 days on/2 days off schedule.

2.3 Exploratory Objectives

1. To assess changes in cell free DNA mutation profile, including mutations in TP53, PIK3CA, ESR1, and RB1 and their correlation with treatment response.
2. To correlate archival tumor mutation profile and RB protein status with treatment response.
3. To assess intrinsic and acquired resistance mechanisms to palbociclib in tumor samples collected at baseline and at progression.
4. To assess changes in serum thymidine kinase activity and correlate with treatment response and absolute neutrophil count.
5. To establish patient derived xenograft (PDX) models from tumor biopsies before treatment and at progression for future studies.
6. To assess baseline and changes in Circulating Tumor Cell (CTC) gene expression profiles and to correlate with treatment response.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

1. Histologically confirmed metastatic ER+ and/or PR+ and HER2- breast cancer who are candidates for palbociclib in combination with either letrozole or fulvestrant per treating physician.

2. Presence of measurable or non-measurable disease by RECIST 1.1 criteria.

3. One prior systemic therapy in the metastatic setting is allowed, but patients who have not had any prior systemic therapies in the metastatic setting are also eligible.
   - Note: patients who were started on endocrine therapy monotherapy as their 1st line or 2nd line systemic therapy in the metastatic setting for no more than 28 days and without clinical progression prior to the initiation of the study drug therapy are allowed to enroll on the study as their 1st line or 2nd line therapy, respectively.

4. At least 18 years of age.

5. ECOG performance status ≤ 2 (see Appendix A)

6. Normal bone marrow and organ function as defined below:
   a. Absolute neutrophil count ≥ 1,500/mcl
   b. Platelets ≥ 100,000/mcl
   c. Total bilirubin ≤ IULN or total bilirubin ≤ 3.0 x IULN with direct bilirubin within normal range in patients with documented Gilbert's syndrome
   d. AST(SGOT)/ALT(SGPT) ≤ 1.5 x IULN (up to 5 x IULN in patients with liver disease)
   e. Creatinine ≤ IULN OR creatinine clearance ≥ 60 mL/min/1.73 m^2 for patients with serum creatinine levels above institutional normal (IULN) (calculated by Creatinine Clearance Estimate by Cockcroft-Gault Equation)

7. Pre- or post-menopausal women are allowed. If pre- or peri-menopausal, concurrent ovarian suppression for pre- or peri-menopausal women is required.

8. Women of childbearing potential must agree to use adequate contraception (hormonal or barrier method of birth control, abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she must inform her treating physician immediately.

9. Able to swallow and retain oral medication.

10. Washout of at least 3 weeks from prior chemotherapy or targeted therapy that induces myelosuppression and recovery of treatment related adverse events to grade 1 or less, with the exception of alopecia, is required prior to the start of palbociclib.

11. 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 therapy with any CDK inhibitor.

2. Currently receiving any other investigational agents.

3. Currently receiving exogenous estrogen replacement therapy (topical vaginal estrogen therapy is allowed).

4. Known brain metastases. Patients with known brain metastases must be excluded from this clinical trial because of their poor prognosis which could affect the evaluation of all-cycle adverse events.

5. A history of allergic reactions attributed to compounds of similar chemical or biologic composition to palbociclib or other agents used in the study.

6. Receiving any medications or substances that are potent inhibitors or inducers of CYP3A isoenzymes within 7 days prior to registration.

7. Clinically significant history of liver disease.

8. A condition that would interfere with enteric absorption.

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

10. Pregnant and/or breastfeeding. Women of childbearing potential must have a negative pregnancy test within 7 days of study entry.

11. Known HIV-positivity on combination antiretroviral therapy because of the potential for pharmacokinetic interactions with palbociclib. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in patients receiving combination antiretroviral therapy when indicated.

3.3 Inclusion of Women and Minorities

Women and members of all races and ethnic groups are eligible for this trial. Enrollment will be restricted to women as breast cancer occurs primarily in women.

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 OnCore 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

5.1 Agent Administration

Agent	Dose	Route	Schedule	Cycle Length
Palbociclib	125 mg	PO	5 days on and 2 days off, starting C1D1	28 days (4 weeks)
Letrozole*	2.5 mg	PO	Daily	
Fulvestrant*	500 mg	IM	Every 14 days for the first 3 doses followed by every 28 days	
Goserelin**	3.6 mg	SC	Every 28 days (Day 1 of each cycle)	
* Letrozole OR fulvestrant will be administered with palbociclib per physician choice. ** Only if pre- or peri-menopausal				

Patients will be instructed to make use of medication diaries (Appendices B and C) to act as records of administration for palbociclib and letrozole (if applicable).

Palbociclib at a dose of 125 mg should be taken by mouth with food on a 5 days on/2 days off schedule (meaning: on Days 1-5, 8-12, 15-19, and 22-26 of each 28-day cycle). If a patient misses a day's dose entirely, she must be instructed not to make it up the next day but just take her regular dose at the next assigned time. If a patient vomits any time after taking a dose, she must be instructed not to retake the dose but resume subsequent dosing at the next assigned time. If a patient inadvertently takes an extra dose during a day, she must be instructed to not take the next day's dose.

Patients who have been on stable doses of palboclib for 6 consecutive cycles can be assessed for retreatment every 2 or 3 cycles to coincide with imaging. These patients must meet retreatment criteria (as described in Section 6.4) and will have labs drawn for retreatment (CBC with differential and CMP) and correlative studies (serum, plasma, cell-free DNA, and circulating tumor cells) to correspond with imaging studies that take place every 2-3 cycles. This allows patients on stable doses of palbociclib to return to clinic for retreatment at greater intervals.

Patients who are receiving letrozole will take it daily by mouth, every day of each 28-day cycle, at a dose of 2.5 mg. If a patient misses a day's dose entirely, she must be instructed not to make it up the next day but just take her regular dose on the next day. If a patient vomits any time after taking a dose, she must be instructed not to retake the dose but resume subsequent dosing on the next day. If a patient inadvertently takes an extra dose during a day, she must be instructed to not take the next day's dose.

Patients who are receiving fulvestrant will receive it at a dose of 500 mg as two 5 mL intramuscular injections (one into each buttock) on Days 1 and 15 of Cycle 1 and then on Day 1 of each cycle thereafter.

Goserelin is given as a subcutaneous injection every 28 days. It is preferred to be given on Day 1 of each cycle, but it may be administered on any day of the treatment cycle to accommodate its specific Q28-day cycle. It will be given to pre- and peri-menopausal women only.

5.2 Evaluability

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.

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

5.3 General Concomitant Medication and Supportive Care Guidelines

While taking palbociclib, patients should be instructed to avoid food or drugs that are known strong CYP3A4 inhibitors or inducers, including grapefruit and grapefruit juice. Please refer to Appendix D 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.

5.4 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 7 days prior to the first dose of palbociclib.

Women of childbearing potential 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.

If a patient is suspected to be pregnant, all study drugs 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 female patient or female partner of a male patient becomes pregnant during therapy or within 90 days after the last dose of palbociclib, the investigator must be notified in order to facilitate outcome follow-up.

5.5 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 indefinitely 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.

5.6 Duration of Follow-up

After coming off treatment, patients will be followed for 30 days to collect adverse events. 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 Hormone Therapy Dose Modifications

No dose adjustments are permitted for fulvestrant or letrozole, but interruptions are allowed at the discretion of the treating physician. Hormonal therapy should continue while holding palbociclib due to palbociclib related AEs.

6.2 Ovarian Suppression Dose Modifications

No dose adjustments are permitted for goserelin.

6.3 Dose Modifications for Palbociclib

Patients will be monitored for toxicity and the dose of palbociclib may be adjusted as indicated in the dose modification table below.

Dose Level	Palbociclib 5 Days On / 2 Days Off
1 (starting dose)	125 mg/day
-1	100 mg/day
-2	75 mg/day
Note that palbociclib dose reduction below 75 mg/day is not allowed	

Dose reduction by 1 and, if needed, 2 dose levels will be allowed depending on the type and severity of toxicity encountered. 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).

Treatment with palbociclib should be permanently discontinued if toxicity has not recovered to grade ≤ 2 within two weeks.

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, and diarrhea 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 PD 0332991 due to adverse events possibly related to study treatment	Decrease by one dose level

6.3.1 Dose Adjustments Due to QTc Prolongation

Any patients who develops new grade 2 or greater ECG QT corrected interval prolonged at any time during the study will need to have the ECG repeated immediately for confirmation.

Grade 2: no adjustments; continue at same dose level
Grade 3 (reversible cause identified and corrected): withhold treatment until QTc ≤ 470 msec, then resume treatment at the same dose level
Grade 3 (no reversible cause identified): withhold treatment until QTc ≤ 470 msec, then decrease palbociclib by one dose level
Grade 4: permanently discontinue palbociclib

6.4 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, hormone therapy 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 despite dose reduction to the lowest dose level, treatment with palbociclib will be permanently discontinued.

7.0 REGULATORY AND REPORTING REQUIREMENTS

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

The Washington University Human Research Protection Office (HRPO) requires that all events meeting the definition of unanticipated problem or serious noncompliance be reported as outlined in Section 7.2.

Pfizer requires that all serious adverse drug experiences (defined as the events described in Sections 7.1.2, 7.1.3, and 7.1.4) be reported as outlined in Section 7.6.

7.1 Definitions

7.1.1 Adverse Events (AEs)

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease.

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

7.1.2 Serious Adverse Event (SAE)

Definition: any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
- A congenital anomaly/birth defect
- Any other experience which, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

7.1.3 Unexpected Adverse Experience

Definition: any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

7.1.4 Life-Threatening Adverse Experience

Definition: any adverse drug experience that places the subject (in the view of the investigator) at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

7.1.5 Unanticipated Problems

Definition:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.1.6 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

7.1.7 Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

7.1.8 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Local IRB pre-approval of all protocol exceptions must be obtained prior to the event. For secondary sites, the Washington University PI will issue approval of the exception, but it must also be submitted to the local IRB with documentation of approval forwarded to Washington University. Washington University IRB approval is not required for protocol exceptions occurring at secondary sites.

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

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within **10 working days** of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.

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

The PI 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 as reportable. (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 a QASMC auditor.

7.4 Reporting Requirements for Secondary Sites

The research team at each secondary site is required to promptly notify the Washington University PI and research coordinator of all reportable events (as described in Section 7.6) 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 either an FDA MedWatch form if required or an institutional SAE reporting form if not). A formal written report must be sent to the Washington University PI and research coordinator within **10 working days** of the occurrence of the event or notification of the secondary site's PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** 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.

7.5 Reporting to Secondary Sites

The Washington University PI (or designee) will notify the research team at each secondary site of all reportable events that have occurred at other sites within **10 working days** of the occurrence of the event or notification of the PI of the event. This includes events that take place both at Washington University and at other secondary sites, if applicable.

7.6 Reporting to Pfizer

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 (those events defined in Sections 7.1.2, 7.1.3, and 7.1.4) that occur during the SAE reporting period (as defined in Section 7.5) in a study subject assigned to receive palbociclib. Such SAEs will be reported using the Serious Adverse Event Report form and Fax Cover Sheet provided by Pfizer. 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.

7.7 Timeframe for Reporting Required Events

Adverse events will be tracked for 30 days following the last day of study treatment.

8.0 PHARMACEUTICAL INFORMATION

8.1 Palbociclib (Ibrance)

8.1.1 Palbociclib Description

Molecular Weight: 447.5
Molecular Formula: $C_{24}H_{29}H_7O_2$

Formulation: Capsules that use common compendial excipients (corn starch, microcrystalline cellulose, sodium starch glycolate, magnesium stearate (nonbovine)) will be used in clinical programs. The capsule shells are manufactured from gelatin NF.

8.1.2 Clinical Pharmacology

Palbociclib is a highly selective inhibitor of Cdk4/cyclinD1 kinase activity (IC_{50} = 11 nM; K_i = 2 nM). Palbociclib 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

To date pharmacokinetic data have been reported for four studies (A5481001, A5481002, A5481003 and A5481004). Final PK data are available from studies A5481001 and A5481002. Pharmacokinetic parameters are available from all 74 patients enrolled in Protocol A5481001 following a single-dose (Day 1 of Cycle 1), and from 51 patients following multiple-dose administration (Day 8 of Cycle 1) of daily doses ranging from 25 to 225 mg of palbociclib. On Day 1, all patients had detectable plasma concentrations of palbociclib at the first measured time point (1 hour) following oral administration. The exposure ($AUC_{(0-10)}$ and C_{max}) increased in a dose-proportional manner over the dose range of 25-225 mg QD following palbociclib administration on Days 1 and 8 of Cycle 1, although some variability (low to moderate) around these doses was observed particularly at the 150 mg QD dose level.

Summary of PD 0332991 Mean and Median Plasma PK Parameters by Dose (Day 1 and Day 8 Data Combined)

Treatment Description (QD)	Study Day	C_{max} [1] (ng/mL)	T_{max} [2] (hour)	$AUC_{(0-10)}$ [1, 3] (ng.hour/mL)
25 mg	1 (n=3)	9.6 (63)	4.0 (4.0-4.0)	58 (51)
	8 (n=3)	15.9 (32)	4.0 (2.0-7.0)	119 (32)
50 mg	1 (n=3)	20.7 (3)	4.0 (4.0-4.3)	134 (5)
	8 (n=3)	35.7 (16)	4.1 (2.0-7.0)	274 (15)
75 mg	1 (n=7)	28.7 (24)	4.0 (4.0-10.0)	199 (20)
	8 (n=6)	58.6 (24)	4.0 (4.0-9.0)	492 (27)
100 mg	1 (n=6)	45.6 (45)	4.0 (2.0-10.0)	332 (34)
	8 (n=6)	71.2 (31)	5.5 (4.0-10.0)	513 (45)
125 mg	1 (n=22)	51.6 (43)	7.0 (2.0-24.4)	299 (44)
	8 (n=13)	86.2 (34)	4.0 (1.0-10.0)	724 (38)
150 mg	1 (n=7)	83.8 (17)	4.0 (4.0-9.8)	633 (9)
	8 (n=6)	161 (44)	7.0 (7.0-10.0)	1342 (42)
200 mg	1 (n=20)	80.8 (35)	5.7 (1.0-10.2)	525 (36)
	8 (n=8)	174 (17)	4.0 (2.0-7.0)	1395 (23)
225 mg	1 (n=6)	104 (58)	4.0 (4.0-7.0)	718 (55)
	8 (n=6)	186 (64)	4.5 (1.0-7.0)	1491 (64)

[1] C_{max} and $AUC_{(0-10)}$: mean (%CV)
[2] T_{max}: Median (Range)
[3] For $AUC_{(0-10)}$, the number of patients on Day 1 for the 100 mg, 125 mg, 150 mg and 200 mg groups were 5, 21, 5 and 19 respectively and on Day 8 for the 75 mg, 100 mg and 125 mg groups were 5, 4 and 12 respectively

Steady-state PK parameters are available for nine patients on Day 14 of Cycle 1 (receiving 200 mg SC 0332991 QD for 2 weeks) and four patients on Day 21 of Cycle 1 (receiving 125 mg QD for 3 weeks). Palbociclib was absorbed with a median Tmax of ~4 hours. The mean palbociclib Vz/F was 3103 L, which is significantly greater than total body water (42 L), indicating that palbociclib extensively penetrates into peripheral tissues. Palbociclib was eliminated slowly; the mean elimination half-life (t½) was 26.5 hours and the mean CL/F was 86.1 L/hour. Palbociclib accumulated following repeated dosing with a median Rac of 2.4, which is consistent with the elimination half-life.

Summary of the Steady-State Mean Plasma PK Parameters on Day 14 (200 mg) and Day 21 (125 mg) Following Oral Administration of PD 0332991 Dose Corrected to 125 mg Dose Level (N=13)

Treatment Description	C_{max}[1] (ng/mL)	T_{max}[2] (hour)	$AUC_{(0-24)}$[1] (ng.hour/mL)	$AUC_{(0-72)}$[1] (ng.hour/mL)	$t_{1/2}$[1] (hour)	CL/F[1] (L/hour)	V_z/F[1] (L)	R_{ac}[2,3]
Dose corrected 125 mg QD (n=13)	104 (48)	4.2 (2-9.8)	1863 (59)	3549 (71)	26.5 (26)	86.1 (50)	3103 (40)	2.4 (1.5-4.2)

[1] mean (%CV)
[2] Median (Range)
[3] For Rac, n=12 ($AUC_{(0-24)}$ was not estimable for Patient 10021099 on Cycle 1, Day 1 in the 200 mg group)
Note: Combined PK parameter data from Day 14 (200 mg) and Day 21 (125 mg) dose corrected to the 125 mg dose level.

Renal excretion of palbociclib was a minor route of elimination with ~1.7% of the drug excreted unchanged in urine over the 10-hour collection period in the 125 mg and 200 mg dose group, combined. The mean renal clearance (CLR) was 6.59 L/hour.

An exploratory evaluation of the circulating metabolites for palbociclib was conducted in plasma samples obtained from patients treated with palbociclib 200 mg QD. Preliminary assessment of the pooled plasma samples on Day 14 of Cycle 1 indicated that the glucuronide conjugate of palbociclib and the lactam of palbociclib were the main metabolites present in plasma. Other metabolites observed were the glucuronide conjugates of hydroxylated palbociclib and the glucuronide conjugate of reduced palbociclib.

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.

Drug-drug interaction between palbociclib and letrozole was evaluated during the Phase 1 portion of a breast cancer study (A5481003). The preliminary data indicate a lack of a potential for drug-drug interaction between palbociclib and letrozole when administered in combination.

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 125 mg, 100 mg, or 75 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 (15-25°C, 59-77°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.

To ensure adequate records, palbociclib capsules will be accounted for as instructed by Pfizer. Unless otherwise authorized by Pfizer, at the end of the clinical trial all drug supplies unallocated or unused by the subjects must be returned to Pfizer or its designee. 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 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

Females of childbearing potential should not handle or administer the study agent unless they are wearing gloves.

8.1.9 Pregnancy

Fertility and teratology studies with PD 0332991 have not been conducted; therefore, safety for pregnant women of childbearing capacity and for the fetus cannot be implied from the existing data. If the drug is used during pregnancy, or if the patient becomes pregnant while receiving this drug, the patient should be apprised of the potential hazard to the fetus.

PD 0332991 caused testicular degeneration in rats and dogs. The incidence and severity was dose related and correlated with decreases in testicular weight in the rat. Testicular degeneration was not reversed after cessation of treatment and progressed in severity in both species. Testicular degeneration produced by PD 0332991 is consistent with Cdk inhibition and alterations in cell cycle kinetics.

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. The decision of effective contraception will be based on the judgment of the principal investigator or a designated associate.

8.1.10 QT Interval

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. In case of QTc prolongation, concomitant conditions such as electrolyte unbalances or use of medications affecting the QT interval should be ruled out or corrected. In case of clinically significant toxicities, PD 0332991 administration should be interrupted and the dose reduced as indicated in clinical protocols.

In Study A5481001 using QTcF, 46 of 73 patients had a maximum increase from baseline of <30 msec and no patient had a maximum on treatment value of ≥500 msec. Notably, one female patient who had received 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. After 7 cycles, the dose was increased to 100 mg QD. The patient remained on treatment for a total of 39 cycles with no cardiac related adverse events. QT data analysis for study A5481002 indicated no clinically significant mean changes with ECGs. Using Fridericia's correction in the A5481002 study, all 17 subjects in the analysis

had a maximum increase from baseline of <30 msec and a maximum post-baseline value for QTc of <500 msec.

8.2 Letrozole (Femara)

8.2.1 Letrozole Description

Letrozole is an aromatase inhibitor indicated for adjuvant treatment of postmenopausal women with hormone receptor positive early breast cancer; extended adjuvant treatment of postmenopausal women with early breast cancer who have received prior standard adjuvant tamoxifen therapy; and first- and second-line treatment of postmenopausal women with hormone receptor positive or unknown advanced breast cancer.

Chemical Name: 4,4'-(1H-1,2,4-Triazol-1-ylmethylne)dibenzonitrile
Molecular Formula: $C_{17}H_{11}N_5$
Molecular Weight: 285.31

8.2.2 Mechanism of Action

Letrozole is a nonsteroidal competitive inhibitor of the aromatase enzyme system; it inhibits the conversion of androgens to estrogens. It 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.

8.2.3 How Supplied

Letrozole tablets are supplied as 2.5 mg tablets. Letrozole is commercially available and will be billed to the patient or her insurance.

8.2.4 Dosage Form and Preparation

Tablets are dark yellow, film-coated, round, slightly biconvex, with beveled edges.

8.2.5 Storage

Store at controlled room temperature (25°C; excursions permitted to 15-30°C).

8.2.6 Method of Administration

Patients should be instructed to take letrozole tablets by mouth with or without food.

8.2.7 Potential Drug Interactions

Letrozole is generally safe to administer with other medicines. However, concomitant use of agents and herbal products that alter ER function are specifically not allowed.

For further information, please refer to the FDA-approved package insert for letrozole.

8.3 Fulvestrant (Faslodex)

8.3.1 Fulvestrant Description

Fulvestrant is an estrogen receptor antagonist indicated for the treatment of hormone receptor positive metastatic breast cancer in postmenopausal women with disease progression following antiestrogen therapy and the treatment of hormone receptor positive, HER2-negative advanced or metastatic breast cancer in combination with palbociclib in women with disease progression after endocrine therapy.

Chemical Name: 7-alpha-[9-(4,4,5,5,5-penta fluoropentylsulphinyl) nonyl]estra-1,3,5-(10)-triene-3,17-beta-diol
Molecular Formula: $C_{32}H_{47}F_5O_3S$
Molecular Weight: 606.77

8.3.2 Mode of Action

Fulvestrant is an estrogen receptor antagonist that binds to the estrogen receptor in a competitive manner with affinity comparable to that of estradiol and downregulates ER protein in human breast cancer cells.

8.3.3 How Supplied

Fulvestrant is supplied as two 5 mL clear neutral glass (Type 1) barrels, each containing 250 mg/5 mL of fulvestrant for intramuscular injection and fitted with a tamper evident closure.

Fulvestrant is commercially available and will be billed to the patient or her insurance.

8.3.4 Dosage Form and Preparation

Fulvestrant, an injection for intramuscular administration, is supplied as 5-mL prefilled syringes containing 250 mg/5mL fulvestrant.

8.3.5 Storage

Refrigerate, 2-8°C. To protect from light, store in the original carton until time of use.

8.3.6 Method of Administration

Fulvestrant will be given as two intramuscular injections (one in each buttock).

8.3.7 Potential Drug Interactions

There are no known drug-drug interactions.

8.4 Goserelin

8.4.1 Goserelin Description

Synthetic decapeptide analogue of GnRH.

Chemical Name or Amino Acid Sequence: [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.
Other Names: Zoladex
Classification: GnRH agonist
Molecular Formula: [$C_{59}H_{84}N_{18}O_{14}$•$(C_2H_4O_2)_x$ where x = 1 to 2.4]
M.W.: 1269
Approximate Solubility: Goserelin is freely soluble in glacial acetic acid. It is soluble in water, 0.1M hydrochloric acid, 0.1M sodium hydroxide, dimethylformamide and dimethyl sulfoxide. Goserelin acetate is practically insoluble in acetone, chloroform and ether [AstraZeneca, Package Insert].

8.4.2 Mode of Action

Goserelin has actions similar to those of naturally occurring GnRH (also known as LHRH). Normally, GnRH is released in a pulsatile manner to maintain levels of gonadotropins. Goserelin, in contrast, is continuously administered, which leads to down-regulation of the GnRH receptor on the pituitary gland and ultimately decreased production of FSH and LH.

8.4.3 How Supplied

Goserelin is supplied as a sterile, biodegradable product containing goserelin acetate equivalent to 3.6 mg of goserelin. Goserelin is designed for subcutaneous injection with continuous release over a 28-day period. Goserelin acetate is dispersed in a matrix of D,L-lactic and glycolic acids copolymer (13.3-14.3 mg/dose) containing less than 2.5% acetic acid and up to 12% goserelin-related

substances and presented as a sterile, white to cream colored 1-mm diameter cylinder, preloaded in a special single use syringe with a 16-gauge x 36 +/- 0.5 mm siliconized needle with protective needle sleeve (SafeSystem™ Syringe) in a sealed, light and moisture proof, aluminum foil laminate pouch containing a desiccant capsule. Studies of the D,L-lactic and glycolic acids copolymer have indicated that it is completely biodegradable and has no demonstrable antigenic potential [AstraZeneca, Package Insert].

Goserelin is commercially available and will be billed to the patient or her insurance.

8.4.4 Storage

Store at controlled room temperature (do not exceed 25°C).

8.4.5 Method of Administration

Goserelin should be administered subcutaneously every 28 days into the anterior abdominal wall below the navel line using an aseptic technique under the supervision of a physician.

8.4.6 Potential Drug Interactions

Goserelin is generally safe to administer with other medicines.

For further information, please refer to the FDA-approved package insert for goserelin.

9.0 CORRELATIVE STUDIES

9.1 Archival Tumor Sample Submission (Mandatory)

All samples should be marked with the patients' study number, initials and date of the sample using an indelible marker. Archival tumor specimens will be requested for research purposes for investigations of predictors of response from all patients pre-registered to the study.

Please submit archival tumor specimens to Dr. Cynthia Ma’s laboratory (address below) within 60 days of registration. Patients enrolled at University of Nebraska could have samples batch shipped to Washington University School of Medicine. If available, archived tumor tissue (from primary breast tumor as well as metastatic tumor, if available) is required for all patients. Tumor blocks will be sectioned and stained for assessment of tumor content. A tumor rich block (from both primary and metastatic tissue, if available) is preferred. Otherwise, 15 to 20 of 10 micron section unstained slides from the tumor rich

area are acceptable. An H&E slide associated with the tumor block or unstained slides is needed as well. Additional tissue may be requested if it is not adequate.

Please include 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 Biopsies (OPTIONAL)

9.2.1 Collection of Specimens

Patients may consent to paired tumor biopsies at baseline and time of progression.

Tissue should be collected as follows:

- First Core (Washington University only) - in 10% formalin to tumor bank
- First Core (Secondary site only) – to be processed into paraffin embedded blocks at local lab/tumor bank per institutional procedures*
- Second Core - immediately frozen in OCT block at bedside
- Third Core (Washington University only) - in DMEM (red medium) fresh to lab for engrafting (to Dr. Shunqiang Li's laboratory)
- Fourth Core - immediately frozen in OCT block at bedside
- Fifth Core (Washington University only) - in DMEM (red medium) fresh to lab for engrafting (to Dr. Shunqiang Li's laboratory)

*Only 1 fixed core (to become 1 block) for each time point will be accepted

9.2.2 Handling of Specimens

Tumor biopsies (with the exception of tumor biopsies collected for PDX models) should be sent to Dr. Watson same day (Washington University patients) or in batch shipments (Secondary site patients).

Mark A. Watson, M.D., Ph.D.
Siteman Cancer Center Tissue Procurement Facility
425 S. Euclid Ave.
Room 5120
St. Louis, MO 63110
Phone: (314) 454-7615
Fax: (314) 454-5525
E-mail: tbank@wudosis.wustl.edu

Tumor biopsies for Human in Mouse Modeling (PDX models) will be placed in cold DMEM High Glucose media, chilled on wet ice and immediately sent to Dr. Shunqiang Li's laboratory for further preparation. The procedure of tissue collection will be handled in a sterile fashion because the tissues will be engrafted into highly immunodeficient NOD/SCID mice. The address is as below:

Shunqiang Li, M.D.
4515 McKinley Research Building
St. Louis, MO 63110
Phone: (314) 747-9311(Lab)
(314) 362-3244 (Office)
(314) 596-8476 (Cell)
Pager: (314) 424-5911 or (314) 508-7804
E-mail: shunqiangli@wustl.edu

9.3 Blood for circulating biomarkers including Serum, Plasma, ctDNA and germline DNA

9.3.1 Collection of Specimens

Blood will be drawn at the following time points for serum, plasma, ctDNA, and germline DNA (only at baseline):

- Baseline
- C1D15
- C2D1
- Every 2-3 months thereafter (to coincide with imaging studies)
- Time of progression

At each time point, the following tubes will be collected:

- 4-5 mL in a serum tube (redtop)
- 8 mL in an EDTA tube (pink or purple top) for plasma
- 8 mL x 2 in a Streck Cell-Free DNA BCT for plasma circulating DNA
- At baseline only, the EDTA tube collected for plasma will also be processed for germline DNA.

9.3.2 Handling of Specimens

Process the serum tube as follows: allow to clot for 30 minutes and then immediately centrifuge at 1200G for 10 minutes at 4°C. The serum should then be stored as 1 mL aliquots at -60°C to -80°C at each site until arrangements are made for shipping to the central site for analysis at the end of the study.

Process plasma tube as follows: mix several times to ensure adequate anticoagulation and place on ice. Deliver tubes to laboratory within 30 minutes of draw and spin at 1000G for 10 min at 4°C. The plasma is aspirated off in 1 mL aliquots and transferred to cryovials to be frozen and stored in LN2 vapor or at -60°C to -80°C.

Process baseline plasma/germline DNA tube as follows: mix several times to ensure adequate anticoagulation and place on ice. Deliver tubes to laboratory within 30 minutes of draw and spin at 1000G for 10 min at 4°C. The plasma is aspirated off in 1 mL aliquots and transferred to cryovials to be frozen and stored in LN2 vapor or at -60°C to -80°C. White blood cell pellets are created with the retaining white blood cells. Germline DNA will be processed from the white blood cell pellets.

Process the Streck Cell-free DNA BCT tubes as follows: mixing several times at room temperature. Do not put on ice. Deliver to tumor bank for further processing and freeze the cell free plasma at -60°C to -80°. Procedures for local processing: Centrifuge the Cell-Free DNA BCT at 1600 x g for 20 minutes at room temperature. Transfer 4.5mL of plasma into three 1.72 mL or one 5.0 mL flip-top microcentrifuge tubes. Centrifuge the 1.7mL or 5.0 mL microcentrifuge tubes at 13000 rpm (~ 16000 x g) for 10 mnutes at 4°C to pellet any remaining cells. Use a P-1000 Rainin Pipette to transfer the supernatant into labeled 2.0 mL or 5.0 mL cryovials. (Avoid contaminating the plasma with any pelleted material, i/e/, buffy coat cells). Place the cryovials into the -60 to -80°C temporary storage location.

Note - that the above sample processing will occur at local laboratories or tumor bank for patients enrolled at University of Nebraska. Samples will be batch shipped to the Siteman Cancer Center Tissue Procurement Facility with address listed below.

Mark A. Watson, M.D., Ph.D.
Siteman Cancer Center Tissue Procurement Facility
425 S. Euclid Ave.
Room 5120
St. Louis, MO 63110
Phone: (314) 454-7615
Fax: (314) 454-5525
E-mail: tbank@wudosis.wustl.edu

9.4 Additional blood draw for CTC (circulating tumor cells):

9.4.1 Collection of Blood

Blood will be drawn at the following time points for CTC:

- Baseline
- C2D1
- Post 2 or 3 months after start of therapy (to coincide with ONLY the 1st imaging assessment of tumor response)
- Time of progression

At each time point, the following tubes will be collected:

- 8ml in a yellow-top ACD tube (provided in kit) to be shipped to UCLA
- (1) additional 8 ml EDTA tube for Washington University patients

Note: Patients who were started on study drug therapy prior to the activation of the protocol version 3 will have blood collected for CTC at the next required timepoints. If all of the pre-progression samples (baseline, C2D1, and with the 1st imaging) were missed, blood will be collected to coincide with the next available imaging timepoint, followed by the blood collection at the subsequent disease progression.

9.4.2 Handling of Specimens

Kit is available for the collection and shipment of blood being shipped to UCLA. Please refer to Appendices E and F for specific collection, handling, and shipping information. Please label specimen with patients' study number, initials and date of the sample using an indelible marker. After mixing, the tube should be placed on ice until shipping overnight via FedEx. Please do not collect blood on Fridays or the day before a Holiday.

Please ship to the address below:

Yazhen Zhu, M.D., Ph.D.
Project Scientist
Department of Molecular and Medical Pharmacology
University of California, Los Angeles
California NanoSystems Institute, Room 4310
570 Westwood Plaza
Los Angeles, CA 90095-1770
Cell: (310) 254 4754
Fax: (310) 206 8975

Additional blood collected for CTC analysis for Washington University patients will need to be transported on ice (after mixing) using a microfluidic device, Parsortix for direct CTC isolation to Dr. Rebecca Aft's laboratory listed below.

Dr. Rebecca Aft Laboratory
Rebecca Aft, M.D., Ph.D.
Clinical Sciences Research Building (CSRB)
Room 6634
St. Louis, MO 63110
Phone: (314) 362-5688

9.4.3 Correlative Study Calendar

Correlative Study	Blood/Tumor	Type of Collection	Volume	Time Point	Process at Site	Temperature conditions for storage/shipping
Archival tumor for research	Primary tumor	Tumor rich block (preferred) or 15-20 10 micron section unstained slides. H&E slide needed as well	N/A	Baseline	N/A	Ambient
Archival tumor for research	Metastatic tumor	Tumor rich block (preferred) or 15-20 10 micron section unstained slides. H&E slide needed as well	N/A	Baseline	N/A	Ambient
Tumor for research (optional)	Tumor	1 core processed into paraffin embedded block, 2 cores frozen in OCT at bedside, 2 cores in DMEM fresh (Wash U patients only)	3 cores secondary sites, 5 cores Wash U only	Baseline and at Time of Disease Progression	NO	Core #1 should be processed into a paraffin embedded block and be stored/shipped at ambient temperature (batch shipments for secondary sites). Cores #2 and #4 should be immediately snap frozen and stored at -60 °C to -80°C (batch shipments for secondary sites). Cores #3 and #5 placed in cold DMEM Glucose media should be chilled on wet ice and immediately taken to Dr. Li's lab (Wash U only)
Serum for research	Whole blood	Clot tube (red)	4-5 mL	Baseline, C1D15, C2D1, every 2-3 months thereafter (to coincide with imaging), time of progression	YES	Stored at -60°C to -80°C (secondary sites will ship in batches)

Plasma for research	Whole blood	EDTA (pink or purple) – the baseline EDTA tube collected for plasma will also be processed for germline DNA	8 mL	Baseline, C1D15, C2D1, every 2-3 months thereafter (to coincide with imaging), time of progression	YES	Stored at -60°C to -80°C (secondary sites will ship in batches)
Whole blood for plasma circulating DNA (ctDNA)	Whole blood	Cell-free DNA BCT (Streck)	8 mL x 2	Baseline, C1D15, C2D1, every 2-3 months thereafter (to coincide with imaging), time of progression	YES	Stored at -60°C to -80°C (secondary sites will ship in batches)
Germline DNA for research	Whole blood	EDTA (pink or purple) – the baseline EDTA tube collected for plasma will also be processed for germline DNA	8 mL	Baseline	YES	Stored at -60°C to -80°C (secondary sites will ship in batches)
Blood for detection of circulating tumor cells	Whole blood	ACD (yellow)	8 mL	Baseline, C2D1, post 2-3 months after start of therapy (coincide with ONLY 1st imaging), time of progression	YES	Shipped overnight to UCLA on ice (avoid Fridays and day before holiday)
Blood for detection of circulating tumor cells (Wash U ONLY)	Whole blood	EDTA (purple)	8 mL	Baseline, C2D1, post 2-3 months after start of therapy (coincide with ONLY 1st imaging), time of progression	YES	Transported on ice (after mixing) to Dr. Aft's lab

10.0 STUDY CALENDAR

Screening/baseline evaluations are to be conducted within 2 weeks prior to start of protocol therapy. Scans and x-rays must be done no more than 4 weeks prior to the start of the protocol therapy. There is a +/-3 day window around each study visit.

	Screening	**Baseline**	**Day 1 of each cycle[8]**	**C1D15**	**C2D1**	**C2D15**	**End of every 2nd or 3rd cycle[8]**	**Time of progression**	**30 day f/u**
Informed consent	X								
H&P, ECOG PS	X		X		X			X	
CBC w/diff	X		X	X	X	X			
CMP	X		X		X				
β-hCG[1]	X								
Imaging	X						X	X	
Palbociclib			Given on a 5 days on / 2 days off schedule for each 28-day cycle						
Fulvestrant[3]			X	X	X				
Letrozole[3]			Daily						
Goserelin[4]			X		X				
Medication diary			Daily, to be returned at the end of each cycle						
AE assessment			X ----------X						
Archival Tissue		X[7]							
Research biopsy (optional)		X						X	
Blood for serum		X		X[5]	X[5]		X[2,5]	X	
Blood for plasma		X		X[5]	X[5]		X[2,5]	X	
Blood for cfDNA		X		X[5]	X[5]		X[2,5]	X	
Blood for germline DNA		X							
Blood for circulating tumor cells		X			X		X[6]	X	

1. Women of childbearing potential only
2. Beginning with the end of Cycle 2 but to coincide with imaging studies
3. Either fulvestrant OR letrozole will be given (MD decision)
4. Goserelin will be given to premenopausal women only
5. Instruct patient take palbociclib/hormonal therapy after blood draw, meaning blood draw needs to happen prior to the day's treatment.
6. Post 2 or 3 months after start of therapy (to coincide with ONLY the 1st imaging assessment of tumor response)
7. Archival tissue should include both primary and metastatic tissue
8. Labs and office visits could be reduced in frequency to q2-3 cycles to coincide with tumor imaging studies in patients on stable doses of palbociclib for at least 6 consecutive cycle

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
Original Consent Form	Prior to registration
On-Study Form	Prior to starting treatment
Treatment Form	Every cycle[1] and end of treatment
Toxicity Form	Continuous
Treatment Summary Form	Completion of treatment
Specimen Collection Form	Per protocol
Follow Up Form	30 Day follow-up
Tumor Measurement Form	Baseline, end of every 2nd or 3rd cycle, and end of treatment

[1]At physician discretion, patients that have been on a stable dose of Palbo for 6 consecutive cycles, may dose in q2-3 cycle intervals to coincide with tumor imaging studies. The treatment data forms will be completed accordingly.

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.

12.0 MEASUREMENT OF EFFECT

12.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 12 weeks. In addition to a baseline scan, confirmatory scans should also be obtained not less than 4 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) (34). Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

12.2 Disease Parameters

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

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

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

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

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

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

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

12.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT: At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the

study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers: Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Cytology, Histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

FDG-PET: While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

- Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
- No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
- FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

12.4 Response Criteria

12.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

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

12.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

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

12.4.3 Evaluation of Best Overall Response

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

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	>4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	>4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once >4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.
Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "symptomatic deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

12.4.4 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

12.4.5 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

12.4.6 Response Review

It is strongly recommended that all responses be reviewed by an expert(s) independent of the study at the study's completion.

13.0 DATA AND SAFETY MONITORING

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, an independent Data and Safety Monitoring Committee (DSMC) 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 DSMC will consist of no fewer than 3 members including 2 clinical investigators and a biostatistician. Like investigators, DSMC members are subject to the Washington University School of Medicine policies regarding standards of conduct. Individuals invited to serve on the DSMC 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.

The Data and Safety Monitoring (DSM) report will be prepared by the study statistician with assistance from the study team, will be reviewed by the DSMC, and will be submitted to the Washington University 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.

Until such a time as the first secondary site activates this protocol, a semi-annual DSM report to be prepared by the study team will be submitted to the QASM Committee beginning 6 months after study activation at Washington University.

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 at https://siteman.wustl.edu/wp-content/uploads/2015/10/QASMC-Policies-and-Procedures-03.31.2015.pdf

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 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
- Completeness of participant 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 Study Description

This is a single arm phase II clinical trial with metastatic HR+ HER2- breast cancer patients treated by palbociclib on a 5 days on/2 days off schedule.

The primary endpoint of the trial is the rate of grade 3 or higher adverse events (AE rate) within the first 29 days (equivalently, 1-AE rate, i.e., the proportion of patients not suffering grade 3 or 4 AEs) The secondary endpoints include AE rate in all cycles, rate of palbociclib dose reduction or interruption or discontinuation, AE profiles, PFS, ORR, and clinical benefit rate.

15.2 Sample Size Calculations

The rate of grade 3 or higher neutropenia in PALOMA -1 and PALOMA – 3 was 54% and 62% respectively. We anticipate the new schedule to have less toxicity leading to around 40%-50% AE rate. The sample size was calculated to test the one-sided null hypothesis that 1-AE (adverse event) rate ≤ 0.38 (i.e., AE rate>0.62) versus the alternative that 1-AE rate ≥ 0.6 (i.e., AE rate<0.4). Enrollment of 47 patients will be required to achieve 90% power (actual power=91.8%) based on one-sample binomial exact test at a 5% level (actual alpha=4.7%). If no grade 3 or higher adverse events were observed on 24 or more patients on the alternative dosing schedule, the alternative dosing will be deemed to show better toxicity profile than standard schedule.

The CBR was estimated as 84.3% (80%~88%) in 1st line and 67% from PALOMA-3 in 2nd line patients. With an estimation that 70% of the enrolled patients are 2nd line, we expect approximately 33 2nd line patients and 14 1st line patients and thus ~22 2nd line patients with ~11 1st line patients with clinical benefit. With 33 2nd line patients, the CBR can be estimated with a 95% two-sided Clopper-Pearson exact CI of 48.5~82.3% given the true CBR is 67% as in PALOMA-3 while the CBR in the 14 1st line patients can be estimated with a 95% CI of 55.5%~97.7% given the true CBR is 84.3%.

15.3 Data Analysis Plan

Patient characteristics and adverse events profiles will be summarized by descriptive statistics. AE rate, ORR, CBR will each be estimated accompanied with 95% confidence interval (CI). PFS will be analyzed by Kaplan-Meier method and 1-year PFS rate will be estimated with 95% CI. All analyses will be conducted among all patients, as well as by subgroups of interest (e.g., line of therapy).

TK1 serum marker will be correlated with absolute neutrophil count along measured time points and tumor response by Pearson or Spearman correlation coefficient as appropriate.

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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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: 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 with food on a 5 days on/2 days off schedule. Take the palbociclib with a glass of water and drink the glass of water in as little time as possible. Swallow the capsules whole and do not chew the capsules.
2. Record the date, the number of capsules taken, and when you took them.
3. If you forget to take your palbociclib 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 capsules taken	Comments
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
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22				
23				
24				
25				
26				
27				
28				

APPENDIX C: 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 daily with or without food, at approximately the same time each day. Take the letrozole with a glass of water and drink the glass of water in as little time as possible. Swallow the letrozole whole and do not chew them.
2. Record the date, the number of tablets taken, and when you took them.
3. If you forget to take your letrozole 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
1				
2				
3				
4				
5				
6				
7				
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APPENDIX D: List of CYP3A4 Strong Inhibitors and Inducers
(http://medicine.iupui.edu/clinpharm/ddis/clinical-table/)

Inhibitors
Indinavir
Nelfinavir
Ritonavir
Clarithromycin
Itraconazole
Ketoconazole
Nefazodone
Lopinavir
Posaconazole
Saquinavir
Telaprevir
Telithromycin
Voriconazole
Grapefruit and grapefruit juice

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

APPENDIX E: OPERATING PROCEDURE FOR CTC COLLECTION

Standard Operating Procedure for Whole Blood Collection
----Kit Instruction for Use (for samples shipped to UCLA)

1 PURPOSE

1.1 Whole Blood Collection is intended for use to perform CTC testing from human blood samples.

2 SCOPE

2.1 Whole Blood Collection Kit is intended for professional use only, by a licensed phlebotomy technician.

3 DEFINITIONS AND ACRONYMS

3.1 N/A

4 RELATED PROCEDURES / FORMS

#	Procedure
SOP0013.2	Instructions for Shipping Human Blood Samples

5 SPECIMEN

5.1 N/A

6 REAGENTS

6.1 N/A

7 SUPPLIES

7.1 **Supplies**

Quantity	Description	Location	Storage upon arrival
1	One FedEx pouch contains one FedEx Express Package Airbill (pre-filled with addresses and priority Overnight checked) , see blood shipment SOP for detail	On top, between the Styrofoam and cardboard shipping boxes	Peel and stick the pouch on top of the cardboard box, retain the sender's copy, affix all the copies of the airbill in the pouch
3	The same FedEx pouch also contains three shipping labels: **1)Biomedical Material, 2)Exempt Human Specimen and 3) Keep refrigerated upon arrival** , see blood shipment SOP for detail	On top, between the Styrofoam and cardboard shipping boxes	Peel and stick these three labels on the side of the cardboard box
2	In 1 small specimen bag (primary container), containing one Yellow top (ACD) blood tube, absorbent pad and	On top of ice gels, in Styrofoam box	RT or 4ºC

	one 50 mL conical tube (secondary container). see Appendix Figure 1 and 2		
3	Three (3) big specimen bags with 3-4 ice gel packs in each specimen bag, see Appendix Figure 4	In Styrofoam box	4ºC
2	The shipping boxes include one Styrofoam box (inside) and one cardboard box (outside), see blood shipment SOP for detail		RT or preferably at 4ºC
1	Blood draw Instruction which is placed in the pouch of small specimen bag		RT

7.2 Equipment and Reagents to be Supplied by User

Quantity	Description
1	BD Vacutainer Safety-Lok Blood Collection Set with Pre-attached holder
1	Elastic band
2	Alcohol antiseptic
1	Gauze sponge
1	Band-aid
1	Instruction for Use

Do NOT use the kit if any damage of the package is observed.

8 QUALITY CONTROL

8.1 Whole Blood Collection Kit components should be obtained in one Biohazard-labeled plastic bag. Do NOT use the kit if any damage of the package is observed.

9 Blood Draw Instructions

Step	Action
	Upon kit arrival
1	Open the cardboard box and locate the FedEx pouch. See blood shipment SOP (instruction) for affixing the Airbill and shipping labels.
2	Open Styrofoam box, locate the small specimen bag which have blood collection tube and 50 mL conical tube. Place these items at room temperature, or keep them in the same Styrofoam box.
3	Store the gel ice packs in 4ºC. Read blood draw instructions located in the pouch of the small specimen bag.
	Blood Draw Procedures
4	Collection and labeling: Draw 1 full tube of blood into the yellow top collection tube. Label the tube with 1) human blood; 2) date (11/17/16) and 3) study number (i.e: W001).
5	Place one blood tube into one 50 mL tube, making sure that the absorbent pad protects the blood tube between blood tube and 50 mL tube. Close the conical

	tube cap tightly. Place the 50 mL tube in the small specimen bag. Seal the specimen bag.
6	Take out all three big ice gel bags from 4ºC. The first big ice gel bag is placed on the bottom of the Styrofoam shipping box. Place the two 50mL conical tubes in the second big ice gel bag which is put above the first ice gel bag. Seal the second bag completely. The third big ice gel bag will be placed on the top of the shipping box covering the two conical tubes. One can open the third big ice gel bag to evenly distribute the small ice gel in the shipping box.
7	Seal the shipping boxes tightly: Seal both inside (Styrofoam) and outside (cardboard) shipping boxes tightly with tape. See the SOP for blood shipment

10 Contact Information

Contact CytoLumina lab at **310-794-1977** or email smalleymatthewd@gmail.com with any questions.

11 Appendix

Figure 1. Picture of labeled tubes in sealed sterile.

Blood Draw Instruction (for customers)

A. Obtain blood draw items in the shipping box and in the hospital.

a1. 1 - BD Vacutainer Safety-Lok Blood Collection Set with Pre-attached holder (not supplied by the kit)
a2. 1 - Elastic band (not supplied by the kit)
a3. 2 - Alcohol antiseptic (not supplied by the kit)
a4. 1 - Band-aid (not supplied by the kit)
a5. 1 - Gauze sponge (not supplied by the kit)
a6. 1 - yellow top blood tube (supplied by the kit)
a7. 1 – 50 mL conical tube with the absorbent pads as the secondary containers for protecting blood tube (supplied by the kit)
a8. 1- small specimen bag. Each 50 mL conical tube is always placed in each small specimen bag
a9. Ice gel packs in three big specimen bags must be stored in refrigerated (4ºC) at least 12 hours before blood draw.

B. Blood Draw Procedure:

b1. Collection and label: Draw 1 full tube of blood into the yellow top collection tube. Label the tube with 1) human blood; 2) date (11/17/16) and 3) study number (i.e: W001)

b2: Place one blood tube into one 50 mL tube, making sure that the absorbent pad protects the blood tube in between blood tube and 50 mL tube. Close the conical tube cap tightly. Place the 50 mL tube in the small specimen bag. Seal the small specimen bag.

b3. Take out all three big ice gel bags from 4ºC. The first big ice gel bag is placed on the bottom of the Styrofoam shipping box. Put the ice gel bag with blood tubes in the middle. Seal the middle big specimen bag tightly. The third big ice gel bag will be placed on the top of the shipping box covering the blood tube/50 mL conical tube.

b4: Seal the shipping boxes tightly: Seal both inside (Styrofoam) and outside (cardboard) shipping boxes tightly with tape. See the SOP for blood shipment

APPENDIX F: OPERATING PROCEDURE FOR CTC HANDLING/SHIPMENT

Standard Operating Procedure (SOP) for Blood Shipping and Handling

1. Whole Blood Shipment kit

Whole Blood shipment kit is intended for use to ship the blood for performing CTC testing from human blood samples.

2. Shipment kit contents

Quantity	Description	Location	Storage upon arrival
1	**One FedEx pouch** contains one FedEx Express Package Airbill (pre-filled with addresses and priority Overnight checked), see Fig 1.	On top, between the Styrofoam and cardboard shipping boxes	Peel and stick the pouch on top of the cardboard box, retain the sender's copy, affix all the copies of the airbill in the pouch
3	The same FedEx pouch also contains three shipping labels: **1)Biomedical Material, 2)Exempt Human Specimen and 3) Keep refrigerated upon arrival,** see Fig 1	On top, between the Styrofoam and cardboard shipping boxes	Peel and stick these three labels on the side of the cardboard box
1	**Blood shipping Instruction** which is placed in the same FedEx pouch	On top, between the Styrofoam and cardboard shipping boxes	RT
2	The shipping boxes include one Styrofoam box (inside) and one cardboard box (outside)		RT or preferably at 4^{o}C

Equipment and Reagents to Be Supplied by User

Quantity	Description
1	Packing tape

Do NOT use the kit if any damage of the package is observed.

3. Shipping Instructions

Biological materials must be packaged according to the triple packaging principle depicted in Figure 1. The three elements of triple packaging include: primary receptacle, leak-proof secondary container, and durable outer container (Fig 2)

3-1: **Shipping kit:**: The items in one shipping kit are:

- In one big specimen bag, contains
  - 1 - yellow top blood collection tube
  - 1 – 50 mL conical tube with the absorbent pads for protecting blood tube
  - 1- small specimen bag. One 50 mL conical tube is always placed in one small bag before or after the blood draw
- Three big specimen bags containing Ice gel packs
- Two shipping boxes include one Styrofoam box (inside) and one cardboard box (outside)

3-2 Open the cardboard box and locate the FedEx pouch. Read the shipping instruction carefully. Peel and stick the pouch on top of the cardboard box. Peel and affix the three labels on the side of the cardboard box: **1)Biomedical Material, 2)Exempt Human Specimen and 3) Keep refrigerated upon arrival,** see Appendix Figure 1

3-3 Use the prefilled FedEx airbill or fill out a new airbillforms as followings:
Item #1 Sender's name and address
Item #3 Recipient's name and address
Item #4 Service: FedEx Priority Overnight
Item #5 Packaging: Other
Item #6: Special Handling, Direct Signature, check "No" for dangerous goods
Item #7: Payment, Bill to _____

3-4 Seal the shipping boxes: Seal both inside (Styrofoam) and outside (cardboard) shipping boxes tightly with tape

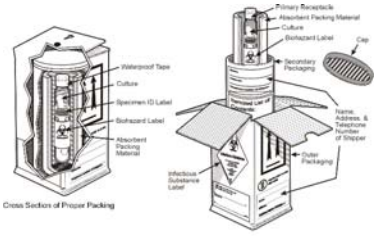
4. Contact Information

Contact CytoLumina lab at **310-794-1977** or email smalleymatthewd@gmail.com to ask any questions.

5. Appendix

Figure 1.

Figure 2.



APPENDIX G: ARCHIVAL TUMOR SPECIMEN SUBMISSION FORM

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

HRPO ID#: 201612098 **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 Medicine ☐University of Nebraska Medical Center

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	Primary or Metastatic
	☐Fixed tissue block ☐Fixed tissue slide			☐Primary ☐Metastatic
	☐Fixed tissue block ☐Fixed tissue slide			☐Primary ☐Metastatic
	☐Fixed tissue block ☐Fixed tissue slide			☐Primary ☐Metastatic
	☐Fixed tissue block ☐Fixed tissue slide			☐Primary ☐Metastatic
	☐Fixed tissue block ☐Fixed tissue slide			☐Primary ☐Metastatic

Processing Notes:
A tumor rich block (from both primary and metastatic tissue, if available) is preferred. Otherwise, 15 to 20 of 10 micron section unstained slides from the tumor rich area are acceptable. An H&E slide associated with the tumor block or unstained slides is needed as well. Additional tissue may be requested if it is not adequate. 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