CLINICAL PROTOCOL

PHASE IB/II OPEN-LABEL SINGLE ARM STUDY TO EVALUATE SAFETY AND EFFICACY OF TUCATINIB IN COMBINATION WITH LETROZOLE AND PALBOCICLIB IN SUBJECTS WITH HORMONE RECEPTOR POSITIVE AND HER2-POSITIVE METASTATIC BREAST CANCER

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PROTOCOL NUMBER: **COMIRB 16-1661, CRITERIUM 17BR01**

PROTOCOL TITLE: PHASE IB/II OPEN-LABEL SINGLE ARM STUDY TO EVALUATE SAFETY AND EFFICACY OF TUCATINIB IN COMBINATION WITH LETROZOLE AND PALBOCICLIB IN SUBJECTS WITH HORMONE RECEPTOR POSITIVE AND HER2-POSITIVE METASTATIC BREAST CANCER

Lead Principal Investigator: Elena Shagisultanova, MD, PhD
University of Colorado Denver

Sponsor: Criterium, Inc.

Investigator Agreement

I have read this protocol and agree that the trial will be carried out in accordance with Good Clinical Practice (GCP) as required by applicable United States (US) laws and applications, including but not limited to United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812).

I will assure that no changes to the protocol will take place without documented approval from the Institutional Review Board (IRB). All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Site Principal Investigator: _____
Print/Type Name

Date: _____ **Signature:** _____

STUDY SYNOPSIS

Concept and Rationale

Dual overexpression of the HER2-neu oncogene and hormone receptors (HR) is found in approximately 10% of all breast carcinomas [1, 2]. These cancers represent a significant therapeutic challenge because of a bi-directional cross-talk between the estrogen receptor (ER) and HER2 pathways leading to accelerated tumor progression and resistance to targeted therapy [3, 4]. Preclinical and clinical data strongly suggest that HER2 overexpression by breast tumors confers an intrinsic resistance to anti-hormonal treatment [2, 5-7], while HR expression is similarly associated with decreased response to HER2-targeted agents [4, 8, 9]. However, at present, endocrine therapy is a standard first line treatment for asymptomatic subjects with metastatic HR-positive (HR+) breast cancer, regardless of the tumor HER2 status [10]. In cell line experiments and murine xenograft models, HER2-targeted agents combined with endocrine therapy showed synergy in suppressing the growth of HR+/HER2+ breast tumors [8, 11]. Several phase III clinical trials combining anti-hormonal therapy with HER2-targeted agents have been performed with modest results [12, 13]. These trials showed statistically significant improvement of progression free survival (PFS) in subjects with HR+/HER2+ tumors receiving endocrine therapy combined with HER2-targeted agents, comparing to endocrine therapy alone. However, these trials were not practice changing because the PFS benefits were small, and no overall survival benefits were demonstrated. Thus, there is a strong pre-clinical and clinical rationale for evaluation of improved, rationally designed drug combinations targeting HR and HER2 pathways in women with HR+/HER2+ breast cancer.

Activation of cell cycle checkpoints (cyclin D1 and CDK4/6 complex) downstream of HR and HER2 pathways plays an important role in the tumorigenesis of HR+/HER2+ breast tumors [14-16]. Mitogenic signaling from both HER2 and HR receptors converges at cell cycle checkpoints and results in the increased Cyclin D1 expression. The frequency of Cyclin D1 amplification and CDK4 gain are higher in HR+/HER2+ breast cancer comparing to other breast cancer subtypes [17]. In mouse models, deletion of cyclin D1 limits the development of HER2-driven tumors [18, 19]. Palbociclib, a CDK4/6 inhibitor, is FDA approved in combination with letrozole in post-menopausal women with HR+/HER2 negative (HER2-) breast cancer, based on improvement of PFS demonstrated in the PALOMA-1 clinical trial [20]. In preclinical studies, palbociclib was active against both luminal A (HR+/HER2-) and luminal B (HR+/HER2+) breast tumors, and synergized with both tamoxifen and anti-HER2 agents (trastuzumab, lapatinib, and TDM-1) providing a potent addition to anti-hormonal and HER2-targeted therapies [14, 16, 21]. Therefore, testing the triple combination of anti-endocrine, CDK4/6 targeted and HER2-targeted agents for HR+/HER2+ breast cancer is a rational approach with potential high impact to the breast cancer field.

We hypothesized that in women with HR+/HER2+ metastatic breast cancer, treatment with a novel HER2-targeted agent tucatinib, combined with a CDK4/6 inhibitor palbociclib and an aromatase inhibitor letrozole will result in synergistic suppression of tumor growth and improved PFS. Tucatinib is a potent oral small-molecule inhibitor highly selective for HER2 receptor tyrosine kinase. With a 500-fold increase in potency for HER2 inhibition compared to closely related kinase EGFR, tucatinib inhibits HER2 signaling while avoiding EGFR-related side effects (skin rash and gastrointestinal toxicity) typical of less selective inhibitors such as lapatinib, neratinib and afatinib [22]. Tucatinib has been tested in multiple phase I clinical trials showing

significant antitumor activity and favorable toxicity profile [23-25]. Notably, tucatinib penetrates through the blood brain barrier and is active in patients with brain metastases.

Tucatinib and palbociclib have largely non-overlapping toxicity profiles. The most common side effects of palbociclib are neutropenia, leukopenia and anemia, while the most common adverse event caused by tucatinib is asymptomatic transaminatis. Non-overlapping toxicity profiles taken together with potentially synergistic mechanism of action of palbociclib and tucatinib provide rationale for combining these agents with letrozole in HR+/HER2+ metastatic breast cancer.

This study is a multicenter, single arm, open-label, run-in phase Ib safety cohort with immediate roll over to a phase II clinical trial that will test the combination therapy of tucatinib with palbociclib and letrozole in subjects with HR+/HER2+ locally advanced unresectable or metastatic breast cancer. As described above, tucatinib has been tested in metastatic HER2+ breast cancer in several phase I/Ib clinical trials, and the maximum tolerated dose (MTD) was determined to be 300 mg PO BID. In this clinical trial, we will first confirm the tolerability of tucatinib given at MTD with standard doses of palbociclib (125 mg daily for 21 days followed by 7 days off treatment) and letrozole (2.5 mg PO daily). In phase II part of this trial, we will expand the testing of this drug combination in subjects with metastatic HR+/HER2+ breast cancer to determine the PFS rate. In addition to the rationale for the synergy of targeting these three pathways simultaneously in this disease setting and its potential for anti-tumor efficacy, we propose this novel combination of three oral agents, if well tolerated, will be highly patient-centered as an effective non-chemotherapy based regimen for treatment of HR+/HER2+ breast cancer.

Phase Ib

Primary objective

- To evaluate safety and tolerability of tucatinib used in combination with palbociclib and letrozole, and to confirm that current RP2D of tucatinib and FDA approved dosing of palbociclib remain the same in the triplet combination

Secondary objectives

- To perform an assessment of pharmacokinetic (PK) properties of tucatinib and palbociclib
- To assess preliminary efficacy of tucatinib used in combination with palbociclib and letrozole

Primary endpoint

- Safety and tolerability of combination therapy as evaluated by standard NCI-CTCAE version 4.03

Secondary endpoints

- Tucatinib and palbociclib plasma concentrations measured during the first and second cycle of therapy
- Assessment of progression free survival (PFS) defined as the time from allocation to the first documented disease progression according to RECIST 1.1 [26] (Appendix C), or death due to any cause, whichever occurs first

- For subjects with brain metastatic disease enrolled in the study, assessment of bi-compartmental PFS in the non-CNS and CNS compartments, defined as the time from allocation to the first documented disease progression according to RECIST 1.1 [26] and RANO-BM criteria [27] (Appendix C), or death due to any cause, whichever occurs first
- Assessment of tumor response – complete response (CR), partial response (PR), or stable disease (SD) of 6 month or longer in duration – by RECIST 1.1 (for subjects with CNS disease by RECIST 1.1 and RANO-BM); overall response rate (ORR) defined as proportion of subjects who had CR or PR; clinical benefit rate (CBR) defined as proportion of subjects with CR, PR and SD; duration of response (DOR) defined as the time from enrollment into clinical trial until objective tumor progression or death whichever occurs first

Phase II part

Primary objective

- To assess efficacy of tucatinib used in combination with palbociclib and letrozole by PFS

Secondary objectives

- To evaluate efficacy of tucatinib used in combination with palbociclib and letrozole by ORR, CBR and DOR
- To evaluate safety and tolerability of the combination therapy

Primary endpoint

- PFS, defined as the time from allocation to the first documented disease progression according to RECIST 1.1, or death due to any cause, whichever occurs first
- For subjects with brain metastatic disease enrolled in the study, assessment of bi-compartmental PFS in the non-CNS and CNS compartments, defined as the time from allocation to the first documented disease progression according to RECIST 1.1 and RANO-BM criteria, or death due to any cause, whichever occurs first

Secondary endpoints

- Assessment of response – CR, PR, or SD of 6 month or longer in duration - according to RECIST 1.1 (for subjects with CNS disease by RECIST 1.1 and RANO-BM); assessment of ORR (CR and PR), CBR (CR, PR and SD), and DOR.
- Incidence, nature and severity of all AEs that occur on or after C1D1 of therapy
- Additional late time point PK assessment of palbociclib and tucatinib

Scientific endpoints / correlative studies for phase IB and phase II

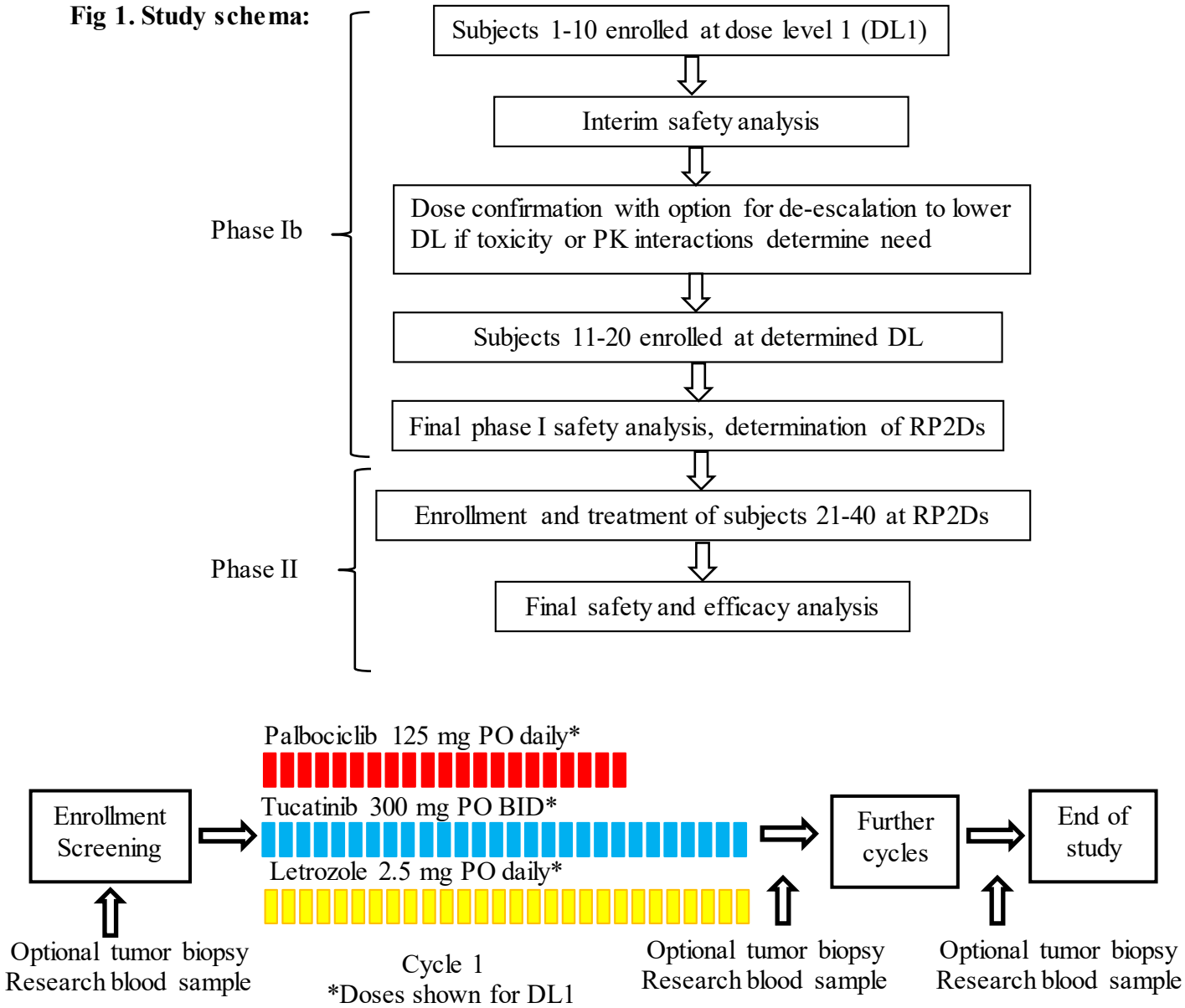
Exploratory assessment of potential biomarkers of resistance and response measured before treatment, after 1 treatment cycle, and at disease progression:

- Assessment of selective markers by IHC in formalin fixed paraffin embedded (FFPE) tumor blocks – such as cyclin E, CDK2, retinoblastoma protein (RB), p16, HER3, total AKT, phospho-AKT, and others as decided by results or advances in the field
- Changes in tumor RNA expression profiles obtained by mRNA Next Generation sequencing in fresh frozen biopsy samples

- Transcript alterations as assessed by mRNA Next Generation sequencing in fresh frozen biopsy samples
- Analysis of tumor specific mutations in circulating tumor DNA (ctDNA) by digital PCR, including activating mutations in estrogen receptor 1 (*ESR1*) and *HER2* genes, and selected mutations revealed by mRNA sequencing, as well as analysis of other circulating markers associated with cancer prognosis or outcomes from treatment
- Correlation between tumor PAM50 subtypes and efficacy of therapy

Study Design

Fig 1. Study schema:



This is a multicenter, single arm, open-label, run-in Phase Ib / roll-over phase II study of tucatinib in combination letrozole and palbociclib in subjects with HR+/HER2+ locally advanced unresectable, or metastatic breast cancer. Premenopausal women may enroll into the study if on treatment with or willing to be treated with standard ovarian suppression. After signing informed consent, completing baseline assessments and meeting all eligibility criteria, the subjects will start treatment. Treatment will be administered in cycles of 28 days each and consist of tucatinib 300 mg PO BID, palbociclib 125 mg PO daily for 21 days followed by 7 days off, and letrozole 2.5 mg PO daily – doses indicated for dose level 1 (DL1). Dose modifications of tucatinib, palbociclib and letrozole will be allowed (as outlined below). Treatment will continue until unacceptable toxicity, disease progression, withdrawal of consent, or study closure. In the absence of clear evidence of clinical or radiographic progression, all efforts should be made to continue treatment until unequivocal evidence of radiologic or clinical progression occurs.

Safety analysis of the combination therapy

Incidence, nature and severity of all AEs that occur on therapy will be analyzed, with particular attention to dose limiting toxicities (DLTs). DLT is a side effect attributed to a study drug or drug combination that is serious enough to meaningfully affect the safety of the study subject. Any drug toxicity listed below is a DLT:

- Any death not clearly due to the underlying disease or extraneous causes
- Grade 3 or higher non-hematologic toxicity
- Symptomatic CHF
- Liver toxicity meeting Hy's law
- Grade 3 neutropenia with fever
- Grade 3 thrombocytopenia with bleeding
- Any grade 4 anemia, neutropenia or thrombocytopenia

All AEs of the specified grades should count as DLTs except those that are clearly and incontrovertibly due to disease progression or extraneous causes. Repeated episodes of grade 3 neutropenia interfering with drug administration and necessitating dose reduction of study medications, or other serious toxicity that, in the opinion of the investigator merits dose reduction of the study drugs, or discontinuation of palbociclib or letrozole according to good clinical practice, will qualify for DLT and should lead to dose modifications of study drugs per protocol.

The following adverse events do not represent DLTs:

- Alopecia of any grade
- Grade 3 nausea/vomiting or diarrhea WITHOUT adequate use of antiemetic or anti-diarrheals that improves with optimal medical management in < 72hrs
- Grade 3 fatigue lasting ≤ 7 days
- Grade 3 rash WITHOUT optimal use of topical corticosteroids or anti-infectives that improves with medical management in <72hrs
- ≥ Grade 3 electrolyte abnormality that lasts < 72 hours that is not clinically complicated, and returns to Grade ≤1 spontaneously or after conventional medical interventions
- ≥ Grade 3 amylase or lipase elevation that is not associated with symptoms or clinical manifestations of pancreatitis

In this study, the proportion of patients experiencing DLTs will be analyzed during the first and second interim safety analyses. Attribution of the DLTs to specific study drug(s) will be performed as described in section 6.2 of the protocol. A DLT experienced by a study subject will always necessitate dose modification and/or interruption of therapy with the study drug(s) for this subject as described in section 6.3 of the protocol. Proportion of patients that experienced DLTs prior to the first interim safety analysis will determine if the study meets pre-specified safety thresholds, and whether de-escalation of the study drug doses to lower dose levels is needed. Analysis of DLTs experienced by all patients enrolled in phase I part of the study will determine the outcomes of the second interim safety analysis, and whether the combination of tucatinib, letrozole and palbociclib demonstrates adequate safety profile allowing to proceed to phase II.

As a signal of lack of safety and tolerability of combination therapy, we will look into the proportion of subjects experiencing DLTs secondary to toxicity of tucatinib and/or palbociclib. The known frequency of DLTs for palbociclib as standard of care therapy when administered in combination with letrozole in the current FDA approved setting are significantly higher than the current dose reduction rates experienced with tucatinib to date. Therefore, the study will be structured to identify an overall clinically meaningful increase in the expected toxicity of palbociclib and/or tucatinib as follows: safety will be considered clinically meaningfully altered if 60% or greater proportion of subjects experience DLTs secondary to the toxicity of palbociclib, or 20% or greater proportion of subjects experience DLTs due to of tucatinib, or 50% or greater proportion of subjects experience DLTs that can be potentially attributed to both tucatinib and palbociclib (Tables 1 and 2).

Table 1. Baseline and expected toxicities of palbociclib and tucatinib

Toxicity attributable to a drug	Baseline rate of DLTs s with single agent therapy	Rate of DLTs triggering safety concern of combination therapy trial
palbociclib	45% [20]	≥60%
tucatinib	10% [22]	≥20%
potentially attributable to both tucatinib and palbociclib		≥50%

Table 2. Safety boundaries for phase Ib part of the trial

Drug	Confidence level	N*	Rate	Upper limit CI	n**
palbociclib	0.9	10	0.6	0.812	8 out of 10
		20	0.6	0.751	15 out of 20
tucatinib	0.9	10	0.2	0.408	4 out of 10
		20	0.2	0.347	7 out of 20
both drugs	0.9	10	0.5	0.760	8 out of 10
		20	0.5	0.694	14 out of 20

*N – total number of subjects; **n – number of subjects experiencing DLTs due to palbociclib and/or tucatinib that is set as a safety boundary

Table 3. Pre-planned dose levels (DLs) for subjects in phase Ib part of the study

DLs	Drug doses	Subjects treated
DL1	tucatinib 300 mg PO BID palbociclib 125 mg PO daily 21 day on, 7 days off letrozole 2.5 mg PO daily	Subjects 1-10 treated at DL1
DL -1	tucatinib 300 mg PO BID palbociclib 100 mg PO daily 21 day on, 7 days off letrozole 2.5 mg PO daily	Subjects 11-20 (if palbociclib toxicity increased)
DL -2	tucatinib 250 mg PO BID palbociclib 125 mg PO daily 21 day on, 7 days off letrozole 2.5 mg PO daily	Subjects 11-20 (if tucatinib toxicity increased)
DL -3	tucatinib 250 mg PO BID palbociclib 100 mg PO daily 21 day on, 7 days off letrozole 2.5 mg PO daily	Subjects 11-20 (if palbociclib and tucatinib toxicity increased, or unattributable toxicity)
DL -4	tucatinib 250 mg PO BID palbociclib 125 mg PO daily 21 day on, 7 days off letrozole 2.5 mg PO daily	Subjects 11-20 (if PKs indicate a significant change to the metabolism of tucatinib)*
DL -5	tucatinib 200 mg PO BID palbociclib 125 mg PO daily 21 day on, 7 days off letrozole 2.5 mg PO daily	Subjects 11-20 (if PKs indicate a significant change to the metabolism of tucatinib)*

*Significant PK changes regardless of noted clinical toxicities

To determine the safety of the triple combination, we will plan to enroll 10 subjects at the baseline starting doses (DL1) of tucatinib 300 mg PO BID, palbociclib 125 mg PO daily 21 days on and 7 days off, and letrozole 2.5 mg PO daily on a 28 day cycle length (Table 3).

After 10 subjects are accrued to the study and complete at least 1 cycle of treatment, we will perform an interim analysis of the safety phase Ib cohort. We anticipate that DLTs will likely be more attributable to palbociclib than tucatinib, but unbiased assessment of the toxicities will be performed. Based on the known rates of dose limiting toxicities to date, as outlined in the table above, the following plan is outlined:

10 subjects enrolled, completed at least one cycle of treatment (28 days) and evaluable for safety analysis:

If there are 7 or fewer subjects experience DLTs due to palbociclib, 3 or fewer subjects experience DLTs due to tucatinib, and 7 or fewer subjects experience DLTs potentially attributable to both drugs, then the interim safety of the Phase Ib cohort will be considered acceptable and accrual will continue to enroll to 20 subjects without change of the starting doses. Otherwise, we will de-escalate to the next dose levels.

Scenario 1: Palbociclib toxicity increased
If 8 out of 10 or greater proportion of subjects experience DLTs attributable to palbociclib, the palbociclib starting dose will be decreased to 100 mg PO daily 21 days on, 7 days off for the subsequent 10 subject in the safety run in phase Ib cohort. Tucatinib starting dose will not be changed (DL -1).

Scenario 2: Tucatinib toxicity increased
If 4 out of 10 or greater proportion of subjects experience DLTs due to tucatinib, we will proceed with a starting dose reduction of tucatinib to 250 mg PO BID and leave palbociclib starting dose unchanged (DL -2).

Scenario 3: Increased toxicity potentially attributable to both drugs:
If there is a lack of clarity or an apparent combination of DLTs that may be attributed to both tucatinib and palbociclib in 8 out of 10 or greater proportion of subjects, then a combined starting dose reduction of tucatinib to 250 mg PO daily and palbociclib to 100 mg PO daily 21 days on and 7 days off will occur (DL -3).

Incorporation of PKs in safety analysis:
Both tucatinib and palbociclib PK data were considered during the first interim safety analysis. Based on the information available prior to phase I of the study, we did not expect significant changes in palbociclib PKs. With palbociclib being a weak time dependent CYP3A4 inhibitor, there was a possibility of tucatinib PK changes. Therefore, the dose levels were designed accordingly: if PKs would be suggesting of a significant change to the metabolism of tucatinib, adjusting to the lower dose levels (DL -4 and DL -5) regardless of noted clinical toxicities was planned.

Based on the existing data for these drugs available prior to initiation of the study, and the non-overlapping toxicity profiles, it was not anticipated that greater than one dose reduction of either or both tucatinib and palbociclib would need to occur to determine the safe combination of drugs to move forward into phase II. If accrual to subjects 11-20 of the phase Ib cohort would demonstrate a level of dose limiting toxicity equal or above the threshold of 15/20 subjects for palbociclib attributable DLTs, 7/20 subjects with tucatinib attributable DLTs, or 14/20 subjects where DLTs were potentially attributable to both tucatinib and palbociclib (Table 2), or if PKs for subjects 11-20 would be suggestive of clinically significant changes in the drug metabolism, then consideration of additional drug specific dose reductions would occur and enrollment in the phase Ib safety cohort would proceed to an additional 10 subjects. Protocol modification and amendment would occur with specific details of the subsequent enrollment and safety analysis planned if this unlikely events were to happen.

Letrozole dose will be kept constant for all study subjects throughout the study. Worsening of estrogen withdrawal symptoms are not anticipated to be increased through the drug combinations nor meaningfully effect the safety assessment of the protocol, as the timing of onset of many AI-based symptoms occurs months to years into the treatment. However, if a subject develops known AI dependent AEs of grade ≥3 (consistent with the definition of DLT), which we expect will occur at a frequency of 4-6% [20], the subject may choose to discontinue AI therapy. Subjects who

discontinue letrozole may opt to remain on protocol if they continue taking both tucatinib and palbociclib, and if it is felt to be in their best clinical interest.

The study has completed the final phase Ib safety analysis on January 31, 2019. Based on the data from the final phase Ib safety analysis, it has been determined that the study did not cross safety thresholds, and full doses of tucatinib, palbociclib and letrozole are reasonably well tolerated. Therefore, the recommended phase II doses (RP2D) were declared as tucatinib 300 mg PO BID; palbociclib 125 mg PO daily 21 days on followed by 7 days off; and letrozole 2.5 mg PO daily (DL1).

However, after the study was re-opened for phase II on January 31-2019, new information on tucatinib became available via Safety Letter from Seattle Genetics sent to investigators on February 13, 2019 ("Re: Safety Communication: Potential Risk of Drug-Drug Interaction"). This letter was to inform the investigators about potential drug-drug interaction between tucatinib and sensitive CYP3A4 substrates (such as palbociclib) based on the results of drug-drug interaction study ONT-380-012. This study demonstrated that in healthy volunteers tucatinib increased the geometric mean of midazolam exposure (AUC) 5.85-fold (90% CI 5.14 – 6.66); therefore, tucatinib belongs to the class of strong CYP3A4 inhibitors. According to the FDA prescribing information, the dose of palbociclib, which is a sensitive CYP3A4 substrate, needs to be decreased to 75mg PO daily 21 days on, 7 days off when palbociclib is used with strong CYP3A4 inhibitors. These findings were discussed with both Pfizer and Seattle Genetics, decision was made to change the dose of palbociclib to 75mg daily for all patients who were on study, and for all newly enrolled patients (as discussed in the Letter to the Investigators from the lead study PI Dr. Shagisultanova dated February 15 2019). Therefore, starting from February 15, 2019, all new patients are enrolled in phase II part of the study at the following drug doses: tucatinib 300 mg PO BID; palbociclib 75 mg PO daily 21 days on followed by 7 days off; and letrozole 2.5 mg PO daily. For all patients who were on study prior to February 15 2019, the dose of palbociclib was changed to 75mg PO daily 21 days on followed by 7 days off (regardless of clinical toxicities), while the doses of tucatinib and letrozole were not changed. Additional PK analysis for palbociclib and tucatinib was planned in patients enrolled in phase II part of the study, to assure safety of the participants.

Dose modifications of the study drugs

Tucatinib will be administered at 300 mg PO BID, which is the current MTD/RP2D when given in combination with trastuzumab, ado-trastuzumab emtansine, or capecitabine. Any grade 3 toxicity related to tucatinib (this excludes bone marrow suppression, hot flashes or other adverse events felt to be clearly related to palbociclib or letrozole) will result in the drug being held until the toxicity returns to grade ≤1. Tucatinib related toxicity must resolve to grade ≤1 within 3 weeks for the subject to remain on study. Then, the subject may resume therapy at 250 mg PO BID. A second and third dose hold for toxicity may occur with the same timeline of 3 weeks for symptom recovery to grade ≤1, then drug may be resumed at 200 mg and 150 mg BID, respectively. If a fourth episode of grade 3 tucatinib-related toxicity occurs, drug should be held and subject removed from study. Once the dose of tucatinib is de-escalated for an individual subject, it will not be increased for that subject during later cycles. At any time if grade 3 toxicity is not resolved to grade ≤1 within 3 weeks, subject will be removed from study. See the full protocol for dose holding and instructions related to cardiac toxicity and liver function abnormalities.

Table 4. Tucatinib dose modifications

Dose Modification for AEs	Dose
Recommended starting dose	300mg PO BID
First dose reduction	250 mg PO BID
Second dose reduction	200 mg PO BID
Third dose reduction	150mg PO BID

Prior to availability of new safety information on tucatinib – palbociclib interaction (prior to February 15, 2019) the following dosing was used for palbociclib:
Palbociclib will be initiated at the FDA approved dose of 125 mg PO daily 21 days on, 7 days off. Dose interruption for known adverse effects, including grade 3-4 bone marrow suppression, should occur per standard therapy (palbociclib full prescribing information brochure) [28]. Palbociclib should be held for any subject who experiences a grade ≥ 3 non-hematologic toxicities clearly related to palbociclib, grade 3 neutropenia with fever, or grade 4 neutropenia, and resumed when symptoms improved to grade ≤2 at the next dose level. Palbociclib should be discontinued if no improvement of symptoms is seen within 3 weeks. Up to two dose reductions of palbociclib are allowed (100 mg, and 75 mg PO daily). Palbociclib dose should not be re-escalated for a subject after a dose reduction is made.

After the information became available to the investigators about potential tucatinib – palbociclib interaction (after February 15, 2019), the following dosing is being used for palbociclib:
Palbociclib will be initiated at the FDA approved dose of 75mg daily 21 days on, 7 days off (FDA approved dose in combination with strong CYP3A4 inhibitors). Dose interruption for known adverse effects, including grade 3-4 bone marrow suppression, should occur per standard therapy (palbociclib full prescribing information brochure) [28]. Palbociclib should be held for any subject who experiences a grade ≥ 3 non-hematologic toxicities clearly related to palbociclib, grade 3 neutropenia with fever, or grade 4 neutropenia, and resumed when symptoms improved to grade ≤2 at the next dose level. Palbociclib should be discontinued if no improvement of symptoms is seen within 3 weeks.

Letrozole will be administered at fixed dose 2.5mg PO daily with no dose adjustments. Premenopausal women enrolled in the study will be required to receive ovarian function suppression (goserelin 3.8 mg subcutaneously every 28 days is the recommended method; however, any standard drug and drug frequency for ovarian function suppression is allowed per discretion of treating physician with Lead PI approval). Letrozole may be held for grade 3 toxicity clearly or potentially attributable to this drug, as determined by the treating physician. Study team should be notified and clear record of the AE according to NCI-CTCAE criteria recorded as for the other two drugs in the combination. When symptoms resolve to ≤ grade 2, the subject may resume letrozole. If symptoms do not resolve to grade ≤2 within 3 weeks, or if the second episode of grade 3 toxicity occurs, letrozole should be discontinued.

Subjects are allowed to stay on the study if either palbociclib or letrozole are discontinued. If both palbociclib and letrozole are stopped for toxicity, subjects will be removed from the study. If tucatinib is discontinued for toxicity, subjects will be removed from the study.

Study Assessments

Baseline Assessments

There will be a screening period of up to 28 days after subjects sign informed consent. During that time, all subjects will undergo standard radiologic imaging for complete assessment of disease to include chest, abdomen and pelvis, as well as bone assessment and appropriate imaging of any other known sites of disease. Recommended imaging includes a diagnostic quality CT scan and bone scan. Brain MRI is not required for neurologically asymptomatic subjects without known brain metastases. Baseline brain MRI is required in subjects with neurological symptoms and/or known brain metastases (CT of the brain will not be allowed, and subjects with known contraindications to undergoing MRI imaging will be excluded from the study). Blood samples for hematology, coagulation, chemistry, and liver function tests will be drawn. All laboratory assessments will be performed using local clinical laboratories. EKG will also be performed, along with assessment of cardiac ejection fraction by MUGA or ECHO. Pregnancy testing (except in women of non-childbearing potential) will be performed within 7 days of the first study treatment. Subjects will have a pre-treatment blood samples drawn to assess for biomarkers of response. Subjects will be asked to undergo optional but highly encouraged tumor core needle biopsy.

Safety Assessments

Subjects will be assessed throughout the study for safety, including physical exam and collection of AEs and laboratory abnormalities performed at a minimum of once every four weeks throughout study treatment and within 30 days after the last dose of study drugs. All laboratory assessments will be performed locally at sites. During cycles 1 and 2, complete blood count and differential will be checked on Day 15 for standard palbociclib monitoring. Assessment of cardiac ejection fraction will be performed by MUGA or ECHO at screening and once every 12 weeks thereafter until study discontinuation, irrespective of dose delays or interruption.

There will be 2 pre-planned interim safety analyses of data obtained from subjects enrolled in phase Ib part of the study prior to proceeding with phase II part. These will occur after 10 subjects are enrolled and have met the safety evaluable time point (completed at least 28 days of treatment) and then after 20 subjects are enrolled and met the safety evaluable time point. The proportion of subjects experiencing DLTs clearly related to tucatinib and/or palbociclib will be determined. Planned starting dose modifications based on these safety assessments are outlined above in "Safety analysis of the combination therapy". The study will be continued to phase II at the planned starting doses or at one of the dose modification levels outlined above if the overall rate of DLTs is $\leq$60% for palbociclib, $\leq$20% for tucatinib and $\leq$ 50% for toxicities potentially attributable to both drugs, and PK analysis is not suggestive of significant changes to the metabolism of the study drugs at a selected dose level. If additional data become available that may affect safety of the participants, doses of the study drugs may be modified accordingly.

Efficacy Assessments

Efficacy assessments will consist of evaluation of all known sites of metastatic or locally advanced unresectable disease. Subjects will undergo standard radiologic imaging for complete assessment of disease to include chest, abdomen and pelvis, as well as bone assessment and appropriate imaging of any other known sites of disease. Recommended imaging includes a diagnostic quality CT scan and bone scan. In addition, all subjects with known brain metastatic disease should have

restaging contrast enhanced MRI of the brain. Imaging studies will be repeated every 8 weeks for the first 24 weeks, and then every 12 weeks thereafter, irrespective of dose holdings or interruptions.

Pharmacokinetic Assessments

PK assessments of blood levels of tucatinib and palbociclib will be performed on Cycle 1 Day 15 and Cycle 2 Day 1 of therapy in 20 participants enrolled in phase Ib part of the study. Plasma samples will be collected to measure levels of tucatinib and its hydroxyl metabolite, as well as levels of palbociclib and, if needed, its metabolites at steady state on Cycle 1 Day 15. Plasma samples will also be collected prior to administration of tucatinib and palbociclib on the first day of Cycles 2 to assess trough levels.

Additional PK assessment will be done in 5 to 10 patients enrolled in phase II part of the study to evaluate the levels of tucatinib and palbociclib. These PKs will be done on Cycle 1 Day 9 and Cycle 2 Day 9. Prior to the first set of PKs, on Cycle 1 Days 1-8, patient will take palbociclib and letrozole; tucatinib will be on hold. Tucatinib will be started per usual study schedule after the first set of PKs is obtained on Day 9. Prior to the second set of PKs, on Cycle 2 Days 1-8, patient will take palbociclib, letrozole and tucatinib (all study drugs) per usual study schedule. On the day prior to PK evaluation (Day 8 of Cycle 1 and Day 8 of Cycle 2), patient should consume high calorie / high fat meal at 8:00PM and take study drugs at 10:00PM. On the next day, plasma samples will be collected to measure PKs at 10, 13, 16 and 19 hrs +/- 10 minutes post dose (8:00AM, 11:00AM, 2:00PM and 5:00PM on Cycle 1 Day 9 and Cycle 2 Day 9). For detailed information on phase II PKs, refer to the LabConnect Manual (PK collection manual) provided by Seattle Genetics, Inc.

Table 5. Schedule of pharmacokinetic assessments

Cycle	Day	Time point
Phase Ib PK assessment		
1	15	0 h (pre-dose) prior to dosing of tucatinib and palbociclib
		0.5 h (±10 minutes) following dosing of tucatinib and palbociclib
		1 h (±10 minutes) following dosing of tucatinib and palbociclib
		2 h (±10 minutes) following dosing of tucatinib and palbociclib
		3 h (±10 minutes) following dosing of tucatinib and palbociclib
		4 h (±10 minutes) following dosing of tucatinib and palbociclib
		6 h (±30 minutes) following dosing of tucatinib and palbociclib
2	1	0 h (pre-dose) prior to dosing of tucatinib and palbociclib
Phase II PK assessment (late PK time points)		
1	1-8	Patient takes palbociclib 75mg and letrozole 2.5mg PO every evening (tucatinib is on hold). On day 8, patient consumes high fat / high calorie meal at 8:00PM and take palbociclib at 10:00PM (leterozole at any time). Patient records timing of study drugs in the diary.
1	9	10 h (±10 minutes) following dosing of palbociclib (8AM)
		13 h (±10 minutes) following dosing of palbociclib (11AM)
		16 h (±10 minutes) following dosing of palbociclib (2PM)
		19 h (±10 minutes) following dosing of palbociclib (5PM)

1	9-28	Post-PKs, patient starts tucatinib, continues on palbociclib and letrozole per study schedule
2	1-8	Patient takes palbociclib, tucatinib and letrozole per study schedule. On day 8, patient consumes high fat / high calorie meal at 8:00PM and take palbociclib and the evening dose of tucatinib at 10:00PM (leterozole at any time). Patient records timing of study drugs in the diary
2	9	10 h (±10 minutes) following dosing of tucatinib and palbociclib (8AM)*
		13 h (±10 minutes) following dosing of tucatinib and palbociclib (11AM)
		16 h (±10 minutes) following dosing of tucatinib and palbociclib (2PM)
		19 h (±10 minutes) following dosing of tucatinib and palbociclib (5PM)

*About 10:00AM patient will be due for the next morning dose of tucatinib, and should take it approximately at this time

Collections of Samples for Assessments of Biomarkers of Response

Subjects will have research blood samples drawn pre-treatment, after 28 days of treatment (±3 days), and after completion of all study treatments at the end of the study. These samples will be obtained for ctDNA analysis, and analysis of other circulating markers associated with cancer prognosis or treatment outcomes. Subjects will be asked to undergo tumor core needle biopsies pre-treatment and at any time during cycle 2. These paired tumor biopsies are optional, but highly encouraged. There will be also optional third biopsy at the time of progression or study withdrawal for other reasons. For detailed information on blood and tissue collection and shipment, please refer to the collection, processing and shipping instructions outlined in the Study Lab Manual. Residual blood and tumor samples left after analysis may be placed in the study biorepository if study subject provides voluntary consent for future research.

End-of-Study Assessments

A final safety assessment including physical examination and laboratory assessments will be required at the time when subjects come off the study, as well as in 30 (±3) days after discontinuation of all study treatments. Subjects will have a research blood sample drawn to assess for the presence of potential biomarkers of response or resistance at the time when they come off the study. Subjects who had progressive disease on therapy will be asked to get an optional tumor biopsy to assess changes in the tumor that determined progression on therapy.

Subject population:

- 20 subjects with locally advanced unresectable or metastatic HR+/HER2+ breast cancer in the phase Ib part of the study.
- 20 subjects with locally advanced unresectable or metastatic HR+/HER2+ positive breast cancer in the phase II part of the study

Study timeline:

- Phase Ib part accrual – 6 month
- Phase II part accrual – 6 month
- Total projected accrual time – 1 year
- Phase Ib primary end-point reporting – 8 month
- Phase II primary end-point reporting – 2.5 years
- Anticipated completion of the study – 2.5 years

This multicenter clinical trial will be conducted through the Academic Breast Cancer Consortium (ABRCC), with the University of Colorado Cancer Center as the lead site. A Protocol Contact Form will be maintained for the study and include a listing of all participating sites and key personnel.

Inclusion criteria:

1. Subjects must have a histologically confirmed diagnosis of HR+/HER2+ locally advanced unresectable or metastatic breast cancer. Estrogen or progesterone receptor positivity is defined by IHC according to the most recent ASCO/CAP guidelines [29]. HER2 positivity is defined by standard of care fluorescence in situ hybridization (FISH) and/or 3+ staining by IHC according to the most recent ASCO/CAP guidelines [30].
2. Measurable and/or evaluable disease per RECIST 1.1 criteria and/or RANO-BM criteria (Appendix C). Bone only disease is allowed.
3. CNS inclusion criteria:
   - Subjects without CNS metastases are eligible. Note: brain imaging is not required for asymptomatic subjects without known brain metastatic disease prior to enrollment into the study
   - Subjects with untreated asymptomatic CNS metastases not needing immediate local therapy in the opinion of investigator are eligible. For subjects with untreated asymptomatic CNS lesions > 2.0 cm by contrast-enhanced MRI, discussion with and approval from the Lead PI is required prior to enrollment
   - Subjects with stable brain metastases previously treated with radiation therapy or surgery are allowed to enroll, provided that they are off corticosteroids or on stable/tapering dose of corticosteroids and stability of CNS metastatic disease for at least 4 weeks has been demonstrated, with the last MRI taken within 2 weeks prior to cycle 1 day 1 of the study. Relevant records of any CNS treatment must be available to allow for classification of target and non-target lesions
4. Age ≥ 18 years
5. ECOG performance status 0-1
6. Life expectancy of more than 6 months, in the opinion of the investigator
7. Study subjects should be post-menopausal women; premenopausal women are eligible if on ovarian suppression, or agreeable to mandatory ovarian suppression. Women of childbearing potential, defined as premenopausal women who are not permanently sterile (i.e., due to hysterectomy, bilateral oophorectomy, bilateral tubal ligation, or bilateral tubal occlusion) are required to have negative pregnancy tests prior to initiation of treatment.
8. Prior treatments:
   - Subjects should have received at least two approved HER2-targeted agents (trastuzumab, pertuzumab, or TDM-1) in the course of their disease
   - Subjects should have had at least 1 line of prior HER2-targeted therapy in the metastatic setting, with the exception of asymptomatic subjects with oligometastatic or bone / soft tissue only disease who, on investigator opinion, are appropriate for a single agent anti-endocrine therapy per NCCN guidelines
   - Subjects who have had up to 2 lines of prior endocrine therapy in the metastatic setting are allowed. Prior adjuvant and/or neoadjuvant endocrine regimens are allowed and not counted towards this limit

9. Adequate organ and marrow function as defined below:
   - Absolute neutrophil count ≥ 1,500/mm^3
   - Platelets ≥ 75,000/mm^3
   - Hemoglobin ≥ 9.0 mg/dL without red blood cell transfusion ≤ 7 days prior to Cycle 1 Day 1 of therapy
   - Total serum bilirubin ≤ 1.5 X upper limit of normal (ULN) except for subjects with known Gilbert's disease, who may enroll if the conjugated bilirubin is ≤ 1.5 ULN
   - AST (SGOT)/ALT (SGPT) ≤2.5 X ULN;
   - Serum creatinine ≤ 1.5 mg/dL
   - International normalized ratio (INR) and activated partial thromboplastin time (aPTT) ≤ 1.5 X ULN unless on medication known to alter INR and aPTT
   - Left ventricular ejection fraction (LVEF) ≥ 50% (as assessed by ECHO or MUGA) documented within 4 weeks prior to first dose of study treatment
   - Serum or urine pregnancy test (for women of childbearing potential) negative ≤ 7 days of starting treatment
10. Ability to understand and the willingness to sign a written informed consent and comply with the study scheduled visits, treatment plans, laboratory tests and other procedures.
11. Subject or legally authorized representative of a subject must provide signed informed consent per a consent document that has been approved by an institutional review board or independent ethics committee (IRB/IEC) prior to initiation of any study-related tests or procedures that are not part of standard-of-care for the subject's disease.

Exclusion criteria:

1. Subjects with previously treated progressing brain metastases are excluded from the study
2. Subjects with known brain metastases and contraindications to undergo contrast MRI imaging of the brain are excluded from the study
3. Pregnancy or breast feeding
4. Current active treatment with an investigational agent
5. Known history of hypersensitivity to aromatase-inhibitor drugs
6. Any toxicity related to prior cancer therapies that has not resolved to ≤ Grade 1, with the exception of peripheral neuropathy, which must have resolved to ≤ Grade 2, and alopecia
7. Previous treatment with lapatinib, neratinib, afatinib, or other investigational EGFR-family receptor tyrosine kinase inhibitor or HER2 tyrosine kinase inhibitor
8. Previous treatment with palbociclib, abemaciclib, ribociclib or other investigational CDK4/6 inhibitors
9. Any systemic anti-cancer therapy (including hormonal therapy), radiation, or experimental agent ≤ 2 weeks of first dose of study treatment
10. Active bacterial, fungal or viral infections requiring treatment with IV antibiotic, IV anti-fungal, or IV anti-viral drugs
11. Known hepatitis B (HBV), hepatitis C (HCV) or human immunodeficiency virus (HIV) infections. Note: pretesting is not required.
12. Inability to swallow pills or any significant gastrointestinal disease which would preclude the adequate oral absorption of medications
13. Use of prohibited medications listed in Appendix D within 3 elimination half-lives prior to first dose of the study treatment

14. Known myocardial infarction, severe/unstable angina, percutaneous transluminal coronary angioplasty/stenting (PTCA), or coronary artery bypass graft (CABG) within 6 month of the first dose of the study treatment
15. Clinically significant cardio-vascular disease, such as ventricular arrhythmia requiring therapy, uncontrolled hypertension (defined as persistent systolic blood pressure > 160 mm Hg and/or diastolic blood pressure > 100 mm Hg on antihypertensive medications), or any history of symptomatic congestive heart failure (CHF)
16. Other severe acute or chronic medical or psychiatric conditions or laboratory abnormalities that may increase the risk associated with study participation or study drug administration, or may interfere with the interpretation of study results, or in the judgment of the investigator would make the subject inappropriate for entry into the study.

Statistical considerations

This is a multicenter, open-label, single arm study to determine safety and efficacy of the tucatinib in combination with palbociclib and letrozole in subjects with HR+/HER2+ locally advanced unresectable or metastatic breast cancer.

The primary endpoint of the phase Ib part of this trial is safety and tolerability of combination therapy as evaluated by standard NCI CTCAE version 4.03. Interim safety analysis will be performed after 10 subjects are accrued to the Phase Ib and again after 20 subjects are accrued to the phase Ib prior to the initiation of phase II part. At these time, we will assess the proportion of subjects that experienced DLTs related to toxicity of tucatinib and/or palbociclib as outlined in "Safety analysis of the combination therapy" and below.

Incidence, nature and severity of all adverse events (AEs) that occur on therapy will be analyzed. Safety will be considered clinically meaningfully altered if 60% or greater proportion of subjects experience DLTs secondary to the toxicity of palbociclib, or 20% or greater proportion of subjects experience DLTs secondary to the toxicity of tucatinib, or 50% or greater proportion of subjects experience DLTs potentially attributable to both drugs (Table 6).

Table 6. Baseline toxicity rates and safety boundaries for palbociclib and tucatinib

Drug	Confidence level	N*	Baseline toxicity rate	Safety boundary rate	Upper limit CI	n**
palbociclib	0.9	10	0.45	0.6	0.812	8 out of 10
		20	0.45	0.6	0.751	15 out of 20
tucatinib	0.9	10	0.10	0.2	0.408	4 out of 10
		20	0.10	0.2	0.347	7 out of 20
both drugs	0.9	10	ND***	0.5	0.760	8 out of 10
		20	ND	0.5	0.694	14 out of 20

*N – total number of subjects; **n – number of subjects experiencing DLTs that is set as safety boundary; ***ND – not determined

To determine the safety of the triple combination, we plan to enroll 10 subjects at the baseline starting doses of tucatinib 300mg PO BID, palbociclib 125mg PO daily 21 days on and 7 days off, and letrozole 2.5mg PO daily on a 28 day cycle length (DL1).

After 10 subjects are accrued to the study and complete at least 1 cycle of treatment, we will perform an interim analysis of the safety Phase Ib cohort. If there are 7 or fewer subjects had DLTs attributable to palbociclib, 3 or fewer subjects had DLTs attributable to tucatinib, and 7 or fewer subjects had DLTs potentially attributable to both drugs, then the interim safety of the Phase Ib cohort will be considered acceptable and accrual will continue to enroll to 20 subjects without change of the starting doses. Otherwise, we will de-escalate to the next dose levels.

Scenario 1: Palbociclib toxicity increased
If 8 out of 10 or greater proportion of subjects had DLTs attributable to palbociclib, we will de-escalate therapy to DL -1.

Scenario 2: Tucatinib toxicity increased
If 4 out of 10 or greater proportion of subjects had DLTs attributable to tucatinib, we will de-escalate therapy to DL -2.

Scenario 3: Unclear or combined attributable toxicity:
If there is a lack of clarity or a combination of toxicities in 8 out of 10 or greater proportion of subjects (DLTs attributable to both tucatinib and palbociclib), doses tucatinib and palbociclib will be de-escalated to DL -3.

Two additional pre-planned dose levels (DL -4 and DL -5) are designed to incorporate the possibility of PK analysis indicating potentially significant changes in the metabolism of tucatinib (regardless of clinical symptoms and DLTs that subjects may or may not experience). Based on the information available prior to phase Ib part of the study, changes in metabolism of palbociclib were not anticipated.

If accrual to subjects 11-20 of the phase Ib cohort demonstrates a level of DLTs equal or above the threshold of 15/20 subjects for palbociclib attributable events, 7/20 subjects with tucatinib attributable events, or 14/20 subjects where toxicities are potentially attributable to both drugs (Table 6), or if PK analysis for subjects 11-20 is suggestive of clinically significant drug interaction, then consideration of additional drug specific dose reductions will occur. This event will need an additional consideration of further dose reductions requiring potential protocol amendment. It could potentially trigger enrollment of additional 10 subjects in the phase Ib safety cohort, or stopping clinical trial for the safety reasons, which will be determined, if this unlikely event should occur, by the Lead PI with the input from the study research team, the sponsor, Pfizer Inc., Seattle Genetics, Inc., and/or the protocol Data Safety Monitoring Committee.

Letrozole dose will be kept constant for all study subjects. Subjects may choose to discontinue letrozole secondary to known aromatase-inhibitor dependent AEs, which we expect will occur at a frequency of 4-6% [20]. However, because these toxicities are not life-threatening, we will not hinge the study safety assessments on letrozole toxicities.

The primary efficacy endpoint for the phase II part is PFS defined as the time from allocation to the first documented disease progression (according to RECIST 1.1 and/or RANO-BM criteria) or death due to any cause, whichever occurs first. If a subject does not have a documented date of progression or death, PFS will be censored at the date of the last adequate assessment. For PFS, Kaplan-Meier quartile estimates along with the 95% CI will be provided.

Phase II study is designed to detect a 50% improvement in PFS from the triple combination of tucatinib, palbociclib and letrozole comparing to historical control. Given significant proportion of heavily pretreated patients with visceral metastases and CNS disease enrolled in clinical trial of tucatinib, palbociclib and letrozole, appropriate historical control should include similar patients. Table 7 below summarizes prior clinical trials of HER2 targeted agents in metastatic breast cancer. Considering characteristics of the patients enrolled in these studies, TH3resa trial of TDM-1 versus physician choice therapy in metastatic HER2+ breast cancer is the most appropriate choice for historical control. TH3resa included patients who received ≥2 HER2-targeted agents for metastatic breast cancer, patients with visceral disease, and patients with CNS disease (although patients with untreated and / or symptomatic CNS disease were excluded). TH3resa demonstrated PSF of 6.2 months in the TDM-1 treatment group. Therefore, considering 50% improvement of PFS comparing to historical control, target PFS for tucatinib, palbociclib and letrozole clinical trial is 9.3 months.

Table 7. Clinical trials of HER2 targeted agents in metastatic breast cancer

Clinical trial	Year of reporting	Prior therapy	Patient characteristics	PFS (months)
PERTAIN trastuzumab, pertuzumab, letrozole ± induction chemotherapy [31]	2017	Front line, no prior therapy for MBC*. Induction chemotherapy was given at the discretion of treating MD	Excluded CNS disease	18
Lapatinib and letrozole [13]	2009	Front line, no prior therapy for MBC	Excluded symptomatic visceral disease and CNS disease	8
Lapatinib and capecitabine [32]	2013	Up to 2 prior trastuzumab containing regimens	Excluded untreated CNS disease	6.8
TH3resa (TDM-1) [33, 34]	2014, 2017	Prior trastuzumab, lapatinib, and taxane; ≥2 HER2-targeted agents for MBC	Excluded untreated and / or symptomatic CNS disease	6.2

Neratinib and capecitabine [35]	2017	No prior lapatinib or capecitabine	Only patients with CNS disease were enrolled	5.5
KAMILLA CNS cohort (TDM-1) [36]	2016	Prior treatment with trastuzumab and taxane	Only patients with CNS disease were enrolled in the CNS cohort	5.5

*MBC – metastatic breast cancer

Using the survival analysis and assuming that the survival time is exponentially distributed, accrual time is 12 months, follow up time is 18 months, and drop out / loss of follow up rate is 5%, the sample size of 40 patients total (20 patients enrolled in phase Ib part, and 20 patients enrolled in phase II part) will allow us to achieve a statistical power of 82% with a one-sided type I error rate of 0.1 to detect 50% improvement in median PFS (from 6.2 month in the historical control to 9.3 months in the current study).

Table 8. Study calendar

Measurement/Treatment	Screening	Treatment Day (Cycle 1) [a]			Treatment Day (Subsequent Cycles) [a]					At completion of the study	
	Days −28 to −1	Day 1	Day 9	Day 15	Cycle 2 Day 1	Cycle 2 Day 9	Cycle 2 Day 15	Cycle 3 Day 1	Cycle 4+ Day 1	End of treatment [l]	30-day post-treatment [m]
Informed Consent and HIPAA Authorization	X										
Inclusion/Exclusion	X	X									
Medical/Cancer History including ER, PR, HER2 status, tumor grade, stage, and prior therapy/dates/response	X										
Physical Examination	X	X[c]			X			X	X	X	X
Vital Signs (HR, temp, BP, HT,WT) [b]	X[b]	X		X	X		X	X	X	X	X
ECOG Performance Status	X	X[c]			X			X	X	X	X
CBC and differential	X	X[c]		X	X		X	X	X	X	X
CMP	X	X[c]			X			X	X	X	X
PT/INR, PTT	X										
Pregnancy Test [d]	X										
Tucatinib, palbociclib and letrozole treatment		--X[e]--		--X--	--X--		--X--	--X--	--X--		
Electrocardiogram (locally read)	X										
Plasma Sample Pharmacokinetics phase I [f]				X	X						
Plasma Sample Pharmacokinetics phase II [g]			X			X					
Radiographic Tumor Evaluation [h]	X							X[h]		X	
ECHO or MUGA [i]	X								X[i]		
Tissue block [j]	X										
Optional tumor biopsy	X				X[n]					X	
Research blood sample	X				X					X	
AE Monitoring [k]		--X--		--X--	--X--		--X--	--X--	--X--	--X--	--X--
Concomitant Medications [k]	X	--X--		--X--	--X--		--X--	--X--	--X--	--X--	--X--

AE – adverse event; ER – estrogen receptor; BP – blood pressure; HER2 – human epidermal growth factor receptor 2; HIPAA – Health Insurance Portability and Accountability Act; HR – heart rate; HT – height; INR – international normalized ratio; RR – respiratory rate; PR – progesterone receptor; PT – prothrombin time; PTT – partial thromboplastin time; CBC – complete blood count; CMP – Complete metabolic profile; WT – weight

Bolded in red color and underlined - non-standard of care interventions

a. Each visit following Day 1 must be completed with ±2 days of the indicated visit day
b. Height needed only at baseline, and not at subsequent assessments
c. Repeated only if more than 72 hours since screening assessments. Assessments must continue to be acceptable if repeated.
d. Women of childbearing potential only, within 7 days prior to the start of treatment.
e. Tucatinib should be held on cycle 1 day 1-8 in patients for whom PKs will be obtained on cycle 1 day 9 (phase II part of the study)
f. PKs for palbociclib and tucatinib will be obtained in 20 subjects enrolled in phase Ib part of the study. PK schedule: PKs on cycle 1 day 15 prior to dosing tucatinib and palbociclib, and in 0.5, 1, 2, 3, 4 and 6 hrs after the dose; PKs on cycle 2 day 1 prior to dosing of tucatinib and palbociclib.
g. PKs for palbociclib and tucatinib will be obtained in 5-10 subjects enrolled in phase II part of the study. These patients should hold tucatinib during cycle 1 days 1-8 (prior to cycle 1 PKs), and resume tucatinib per protocol on cycle 1 day 9, after PKs are obtained. Patients should take all study drugs per protocol during cycle 2 days 1-8. On day 8 of cycle 1 and cycle 2 (a day prior to PK analysis) a patient should consume high calorie / high protein meal at 8:00PM and take study drugs at 10:00PM. On day 9 of cycle 1 and cycle 2, PKs will be obtained at 10, 13, 16, and 19 hrs after the drug dose (at 8AM, 11AM, 2PM and 5PM).
h. Imaging studies must be done within 28 days prior to entering study and may include CT C/A/P, NM bone scan, and appropriate imaging to assess any other known site of disease. Subjects with known CNS disease should get contrast-enhanced MRIs of the brain. Intervals of scans will be every 2 months for the first 6 cycles (at day 26 ± 3 days on cycles 2, 4, and 6), and then every 3 months for all subsequent cycles (at day 26 ± 3 days on cycles 9, 12, 15, etc.). If cycles are delayed for any reason, then perform radiographic assessment of metastatic disease in 8 weeks (±3 days) from the previous scan for the first 24 weeks of the study, and in 12 weeks (±3days) afterwards.
i. ECHO/MUGA testing be done at baseline, and repeated every 12 weeks after cycle 1 day 1 of the study (on day 26 ± 3 days of cycle 3, 6, 9, 12 and etc.). Whichever testing modality is chosen in screening should be used for all subsequent cardiac assessments throughout the study for comparison. If cycles are delayed for any reason or there is an interim assessment, then perform assessment in 12 weeks (±3 days) from previous ECHO or MUGA.
j. Collection of primary breast tumor and metastatic tumor archival tissue block or slides will be initiated after enrollment (please refer to the Study Lab Manual for details).
k. AEs and concomitant medications are monitored continually while on study. Subjects are instructed to call with AE symptoms and change in concomitant medications between scheduled visits
l. End of treatment visit shall occur at the time or within 7 days after the subject discontinues treatment with study drug.
m. 30-day post-treatment safety visit shall occur in 30±3 days after the subject discontinued treatment with study drug.
n. Optional tumor biopsy can be performed at any time during cycle 2.

Contents

1 CONCEPT AND RATIONALE

1.1 Hormone receptor-positive and HER2-positive breast cancer

Breast cancer is the most frequently diagnosed cancer, excluding skin malignancies, and a second leading cause of cancer death after lung cancer in women in the United States [37]. Approximately 20% of breast cancers overexpress the human epidermal growth factor receptor 2 (HER2), a transmembrane tyrosine kinase receptor mediating cell growth, differentiation, and survival [38]. HER2-positive (HER2+) breast tumors are more aggressive and historically have been associated with poorer outcomes compared to HER2-negative (HER2-) tumors, although the introduction of HER2-targeted therapies allowed for significant improvements in survival of subjects with HER2+ breast cancer [39-41]. Approximately half of HER2+ breast tumors overexpress hormone receptors (HR) [1, 2]. These cancers represent a significant therapeutic challenge because of a bi-directional cross-talk between estrogen receptor (ER) and HER2 pathways leading to accelerated tumor progression and resistance to targeted therapies [3, 4]. Preclinical and clinical data strongly suggest that HR expression is associated with decreased response to HER2-targeted agents [4, 8, 9], while HER2 overexpression confers intrinsic resistance to anti-hormonal treatments [2, 5-7].

1.2 HR expression confers resistance to HER2-targeted therapies

In HR+/HER2+ breast cancer cell lines, ER signaling and ER transcriptional activity are upregulated following trastuzumab and lapatinib treatment, and ER functions as the key escape/survival pathway leading to reduced sensitivity to HER2-targeted agents [8, 42, 43]. Retrospective analysis of clinical data showed that only 15% of HR+/HER2+ subjects achieved pathologic complete response (pCR) with neo-adjuvant transtuzumab and chemotherapy treatment, while pCR rate was almost 2 time higher (29%) in HR-/HER2+ subjects [44]. Data from prospective neo-adjuvant clinical trials such as NeoSphere [45], NeoALTTO [46], GeparQuinto [47], NOAH [48], ACOSOG Z1041 [49], and TBCRC 006 [50] consistently demonstrated lower pCR rates in HR+/HER2+ subjects regardless of the type of HER2-targeted agents used. Preclinical and clinical studies in HER2+ metastatic breast cancer confirmed that expression of HR was associated with reduced responsiveness to trastuzumab [8, 9], and addition of endocrine therapy to the HER2-targeted agents led to modest progression free survival (PFS) benefits in a few trials [12, 13].

1.3 HER2 overexpression is associated with resistance to anti-hormonal therapy

Crosstalk between ER and HER2 is a major determinant of resistance to endocrine therapy. In preclinical experiments, HR+ breast cancer cell lines that are sensitive to tamoxifen acquire tamoxifen resistance after transfection with HER2 oncogene [5, 7]. Analysis of tumor samples from postmenopausal patients with stage II and III HR+ breast cancers treated on two independent neoadjuvant endocrine therapy trials showed that HR+/HER2+ tumors have statistically significantly higher histologic grade, higher Ki-67, and significantly less suppression of Ki-67 after treatment with AI or tamoxifen compared with HR+/HER2- tumors. These tumors display continued estrogen-independent proliferation despite ongoing endocrine therapy [51]. Results of two adjuvant therapy clinical trials (Breast International Group 1-98 study and the Arimidex or Tamoxifen Alone or in Combination (ATAC) study) revealed that HER2+ status is associated with a significantly higher relapse rate, regardless of whether the hormonal therapy administered was an AI or tamoxifen in the adjuvant setting [52, 53]. Studies in metastatic breast cancer also showed that patients with HR+/HER2+ tumors have decreased responses to tamoxifen and aromatase

inhibitors (AIs) [51, 53-56]. In patients with HR+/HER2+ tumors, median time-to-progression (TTP) on first-line endocrine therapy was 2 times shorter, comparing to patients with HR+/HER- tumors (5.5 months versus 11.2 months; P<0.001) [56].

At present, endocrine therapy may be used as a standard first line treatment for asymptomatic patients with metastatic HR+/HER2+ breast cancer [57]. However, as described above, this therapy is frequently ineffective because of intrinsic resistance to endocrine agents conferred by overexpression of HER2 oncogene [51, 53-56].

1.4 Combining anti-hormonal and HER2-targeted agents in metastatic breast cancer

Preclinical modeling in breast tumor cell lines and murine xenografts demonstrated synergy of HER2-targeted agents combined with endocrine therapy in suppressing growth of HR and HER2 positive breast tumors [8, 11]. However, translation of these exciting preclinical results into human clinical trials has been disappointing. Results of two randomized phase III clinical trials combining anti-hormonal therapy with HER2-targeted agents have been reported [12, 13]. TAnDEM trial evaluated the benefit of adding trastuzumab to anastrozole as a front-line therapy in patients with HR+/HER2+ metastatic breast cancer. Median PFS was 4.8 months for the combination group versus 2.4 months for the anastrozole monotherapy group, with a hazard ratio of 0.63 (P=0.016; 95% CI, 0.47 to 0.84). In patients with centrally confirmed HR+ tumors, median PFS was 5.6 vs 3.8 month in the trastuzumab plus anastrozole and anastrozole alone arms, respectively (P=0.006). The ORR was significantly higher with the combination treatment compared with anastrozole alone (20.3% *v* 6.8%; P=018). The clinical benefit rate (CBR), defined as rate of complete response (CR), partial response (PR), and stable disease (SD) 6 months or longer in duration, was also significantly higher in patients in the combination arm compared with those in the anastrozole arm (42.7% *v* 27.9%; P=0.026). No statistically significant improvement in overall survival (OS) was demonstrated, although median OS was numerically longer in patients receiving trastuzumab and anastrozole compared with patients receiving anastrozole monotherapy (28.5 *v* 23.9 months; P=0.325) [12].

Similarly, in the EGF30008 study, anti-HER2 tyrosine kinase inhibitor lapatinib was combined with letrozole and compared with letrozole plus placebo in patients with HR+ metastatic breast cancer. In the HER2+ subgroup, the addition of lapatinib reduced risk of disease progression, with a hazard ratio of 0.71 (P=0.019; 95% CI, 0.53 to 0.96) and median PFS of 8.2 versus 3.0 months. The ORR was also higher in the combination therapy group (28% *v* 15%; P=0.021). CBR was significantly greater for lapatinib plus letrozole (48% *v* 29%; odds ratio 0.4; 95%CI, 0.2 to 0.8; P=0.03). Again, these benefits did not translate into an improvement in median OS (33.3 *v* 32.3 months) [13].

Although TAnDEM and EGF30008 clinical trials showed statistically significant improvement of PFS in patients with HR+/HER2+ tumors with addition of HER2-targeted agents to endocrine therapy, these trials were not practice changing because PFS benefits were small, and no benefits in OS were demonstrated. What is not known is whether these results were due to limitations of specific HER2-targeted agents (trastuzumab, lapatinib), inherent endocrine resistance that was not counteracted by anti-HER2 therapy alone, other resistance mechanisms occurring, or a combination of all of these factors. New, rationally designed combinations of HER2-targeted and anti-hormonal agents are warranted.

1.5 CDK4/6 inhibitors synergize with anti-hormonal and HER2-targeted agents

Activation of cell cycle checkpoints – cyclin D1 and cyclin dependent kinases (CDKs) 4 and 6 – plays a critical role in the tumorigenesis of HR+/HER2+ breast cancer [14-16]. Mitogenic signaling from HER2 and HR receptors converges at cell cycle checkpoints and results in the increased cyclin D1 expression. In mouse models, cyclin D1 activity is critical for the development of breast tumors, and deletion of cyclin D1 gene limits tumor growth driven by HER2 oncogene [18, 19]. Cyclin D1 and CDK4 are commonly amplified in HR+/HER2+ breast cancer, and the frequency of these amplification events is higher comparing to other breast cancer subtypes [17].

Inhibition of cyclin D1 - CDK4/6 complex emerged as a promising therapeutic strategy in luminal breast cancer. In a pivotal study of CDK4/6 inhibitor palbociclib, Finn and colleagues [14] compared baseline gene expression profiles from breast cancer cell lines highly sensitive and resistant to palbociclib. HR+ cell lines, including those with HER2 amplification, were the most sensitive, and there was a significant overlap between the gene expression profiles associated with palbociclib sensitivity and that which distinguishes a luminal breast cancer subtype [14].

Palbociclib was FDA approved in patient with HR+ metastatic breast cancer based on the results of PALOMA-1 randomized phase II clinical trial, where it showed marked improvement in median PFS in women who received palbociclib and letrozole versus letrozole alone (26.1 versus 7.5 months) [20]. Additionally, palbociclib demonstrated remarkable activity in the second line metastatic setting in combination with fulvestrant in the PALOMA-3 clinical trial, resulting in more than doubling of median PFS (9.2 months palbociclib with fulvestrant vs 3.8 months placebo with fulvestrant; HR 0.42; P <0.001) [58]. In preclinical studies, palbociclib was active against both luminal A (HR+/HER2-) and luminal B (HR+/HER2+) breast tumors, and synergized with both tamoxifen and anti-HER2 agents (trastuzumab, lapatinib, and TDM-1) providing a potent addition to anti-hormonal and HER2-targeted therapies [14, 16, 21].

The ability of CDK4/6 inhibitors to synergize with HER2-targeted agents and overcome *de-novo* and acquired endocrine resistance may prove extremely useful in patients with HR+/HER2+ breast cancer. Several clinical trials are ongoing in Europe to test combinations of CDK4/6 inhibitors with endocrine therapy and HER2-targeted agents. A phase II clinical trial of palbociclib in combination with trastuzumab with and without letrozole (PATRICIA) is recruiting patients in Spain (NCT02448420; www. clinicaltrials.gov). A trial of neo-adjuvant palbociclib added to trastuzumab, pertuzumab and fulvestrant is ongoing in Italy (NCT02530424). As of October 2016, no active clinical trials existed in the US that would be testing the triple combination of anti-endocrine, CDK4/6 targeted and HER2-targeted agents for HR+/HER2+ breast cancer, making this proposal both novel and with potential high impact to the breast cancer field.

1.6 Novel HER2-targeted small molecule inhibitor tucatinib synergizes with palbociclib and fulvestrant in tumor cell lines

In our laboratory, we performed cell proliferation assays titrating novel HER2 tyrosine kinase inhibitor tucatinib, palbociclib and fulvestrant in BT474 and MDA-MB-361 HR+/HER2+ tumor cell lines. In our experiments we used fulvestrant instead of letrozole, as letrozole has no activity in tumor cell line models, unless the cells are transfected with the aromatase gene. Results showed productive interaction between tucatinib, palbociclib and fulvestrant in reducing cell proliferation and improving tumor cell killing (Fig. 1).

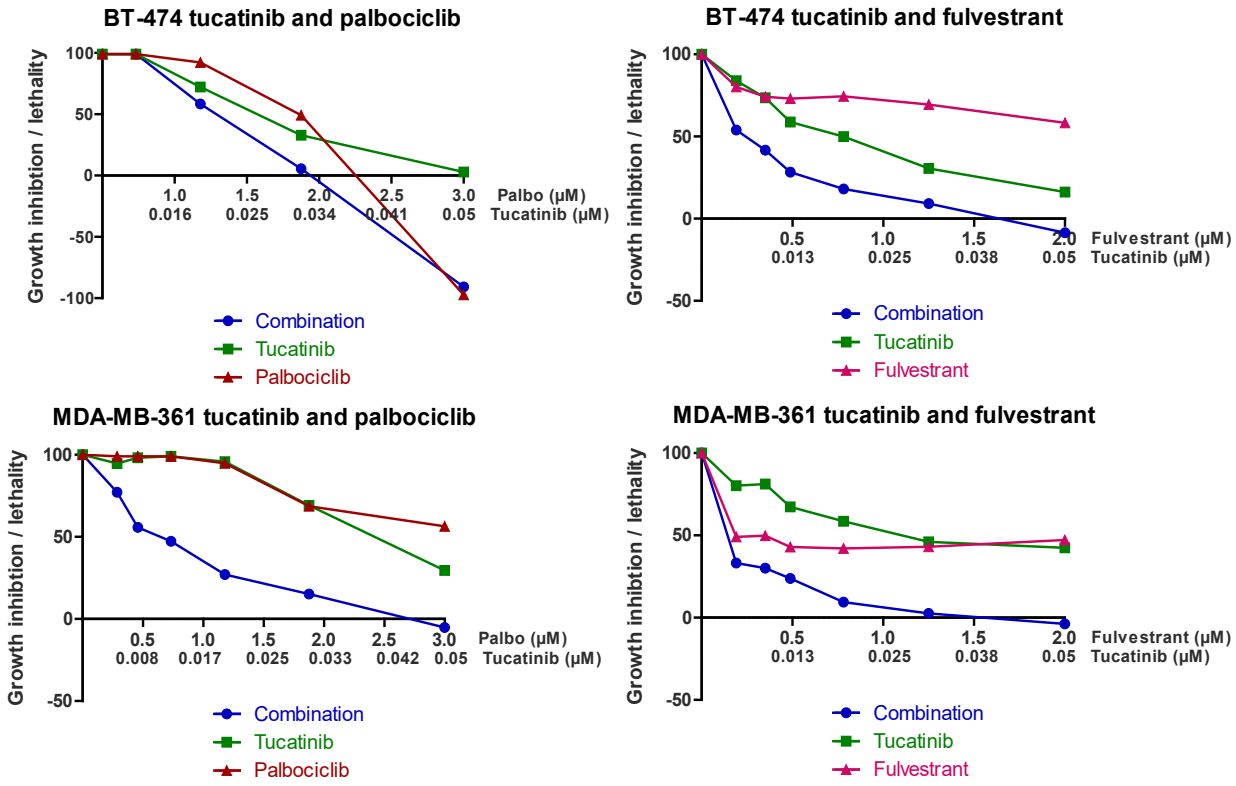


Fig. 1. Titration of palbociclib, tucatinib and fulvestrant in HR+/HER2+ tumor cell lines

1.7 Rationale for combining HER2-targeted agents with anti-hormonal agents and CDK4/6 inhibitors in HR+/HER2+ breast cancer

We hypothesized that in subjects with HR+/HER2+ metastatic breast cancer, treatment with a novel HER2-targeted agent tucatinib, combined with an aromatase inhibitor leterozole and CDK4/6 inhibitor palbociclib will result in synergistic suppression of tumor growth and significant improvement in progression free survival (PFS). To test this hypothesis, we proposed a phase Ib/II clinical trial.

Tucatinib is a novel potent oral small-molecule inhibitor highly selective for HER2 receptor tyrosine kinase [25]. One of the key features of tucatinib is its potency and selectivity for HER2 compared to the closely related kinase EGFR. With a 500-fold increase in potency for HER2 inhibition compared to EGFR, tucatinib has the potential to inhibit HER2 signaling while avoiding known EGFR-related side effects (severe skin rash and gastrointestinal (GI) toxicity) [22]. Tucatinib has been tested in breast cancer subjects in several phase I clinical trials showing significant antitumor activity and very favorable toxicity profile [23-25, 59].

This clinical trial will test combination therapy of tucatinib in the dose of 300 mg PO BID with palbociclib in a standard dose of 125 mg PO daily for 21 consecutive days followed by 7 days off treatment and letrozole 2.5 mg PO daily in subjects with HR+/HER2+ metastatic breast cancer. In this clinical trial, we will first determine that the recommended dose of tucatinib combines with the FDA approved doses of palbociclib and letrozole with no unexpected overlapping toxicities that would require dose decreases for the combination. This will be followed by testing our drug combination in the expansion cohort of subjects with metastatic HR+/HER2+ positive breast

cancer, to further delineate the safety and assess efficacy of tucatinib combined with palbociclib and letrozole.

Tucatinib and palbociclib have largely non-overlapping toxicity profiles. Most common serious side effects of palbociclib are neutropenia, leukopenia and anemia, while most common serious adverse events (SAEs) caused by tucatinib are liver function test abnormalities. Non-overlapping toxicity profiles taken together with potentially synergistic mechanism of action of palbociclib and tucatinib provide rational for combining these agents with letrozole in HR+/HER2+ metastatic breast cancer subjects. In addition to the rationale for the synergy of targeting these three pathways simultaneously in this disease setting and its potential for anti-tumor efficacy, we propose this novel combination of three oral agents, if well tolerated, will be highly subject-centered as an effective, all orally administered, non-chemotherapy based regimen for treatment of HR+/HER2+ metastatic breast cancer.

2 STUDY DRUGS

2.1 Tucatinib

2.1.1 Mechanism of action

Tucatinib [ARRAY-380, ONT-380] is an orally available, reversible HER2 small molecule tyrosine kinase inhibitor that is being developed as a novel treatment for HER2+ breast cancer. One of the key features of tucatinib is its potency and selectivity for HER2 compared to the closely related kinase EGFR. With a 500-fold increase in potency for HER2 inhibition compared to EGFR, tucatinib has the potential to inhibit HER2 signaling while avoiding known EGFR-related side effects, in particular, severe skin rash and gastrointestinal toxicity [22]. This unique feature differentiates tucatinib from other HER2 inhibitors, including neratinib, afatinib and lapatinib, which inhibit HER2 and EGFR with similar potency and are associated with side effects of EGFR inhibition.

2.1.2 *In vitro* biochemistry and cell biology

Using a panel of 223 protein kinases, the only enzymes inhibited by tucatinib by 75% when tested at either 1 or 10 μM were members of the ErbB kinase family (HER2, EGFR, and HER4). Further analysis of this family of kinases using biochemical assays demonstrated tucatinib inhibited HER2 with an IC_{50} (half maximal inhibitory concentration) of 22 nM and was less active against EGFR (IC_{50}=94 nM) and HER4 (IC_{50}=370 nM). The selectivity of tucatinib for HER2 is better exemplified in assays designed to measure the inhibition of HER2 and EGFR auto-phosphorylation using tumor cell lines. In the HER2 overexpressing cell line BT-474, tucatinib inhibited the phosphorylation of HER2 with an IC_{50}=8 nM and inhibited the phosphorylation of Akt, a downstream effector of HER2, with an IC_{50}=3 nM. Consistent with the potent inhibition of HER2 phosphorylation in this cell line, tucatinib blocked the proliferation of BT-474 cells with an IC_{50}=11 nM. In contrast, tucatinib only weakly inhibited the phosphorylation of EGFR in the overexpressing cell line A431, producing an IC_{50}=4000 nM and only inhibited proliferation of A431 cells at drug concentrations greater than 1 mM. These data demonstrate a high degree of selectivity (500-fold) for HER2 relative to EGFR and are consistent with the idea that tucatinib has the capacity to block HER2 signaling without the contributing toxicities of EGFR inhibition.

2.1.3 *In vivo* pharmacology

Tucatinib has been studied in a variety of *in vivo* HER2+ tumor models. Tucatinib at doses of 25, 50 and 100 mg/kg significantly inhibited tumor growth in subcutaneous tumor models derived from BT-474 HR+/HER2+ breast tumor cell line. In the BT-474 model, tumor growth inhibition by tucatinib was 81% at 50 mg/kg and was greater than the inhibition observed with trastuzumab (20 mg/kg). Additionally, tucatinib was evaluated in combination with trastuzumab or docetaxel in subcutaneous HER2+ tumor models. The drug doublets were well tolerated and more efficacious than any single agent. When tucatinib was combined with trastuzumab (20 mg/kg) in the BT-474 model, the anti-tumor activity exceeded either drug when dosed as a single agent. Similarly, the combination of tucatinib with docetaxel (10 mg/kg) in the BT-474 model was also more effective than either single agent. To determine the potential of tucatinib in treating brain metastatic HER2+ breast cancer, tucatinib was tested using BT-474 intracranial tumor implants. Treatment of mice with tucatinib in the dose of 75 mg/kg BID conferred a significant survival benefit compared with lapatinib in the dose of 50 mg/kg BID, or neratinib in the dose of 40 mg/kg QID when each drug was dosed at the MTD in the BT-474 model. Taken together, these data support the hypothesis that tucatinib is effective for treatment of HER2+ breast cancer that has metastasized to the brain.

2.1.4 Non-clinical safety

Good Laboratory Practice toxicology, safety pharmacology, and tolerability studies with tucatinib have been conducted in rats and cynomolgous monkeys to extrapolate the safety of administering the drug to humans. Taken together, these studies have demonstrated that tucatinib has a satisfactory safety profile and presents limited risk in humans. Doses of 30 mg/kg BID in rats and 10 mg/kg BID in monkeys were well tolerated over the 28-days testing period. Safety pharmacology studies were conducted to examine the effect of tucatinib on cardiovascular function using *in vitro* human ether-a-go-go related gene (hERG) inhibition assays, and by *in vivo* telemetry using cynomolgous monkeys. Titration of tucatinib in the hERG assay produced an IC_{50}=13.5 μM, and there were no significant effects noted in mean arterial blood pressure, heart rate or electrocardiogram (ECG) waveforms or in QT and corrected QT (QTc) measurements in the *in vivo* study. There were also no effects noted in gastrointestinal secretion and propulsion, neurobehavioral, or respiratory function studies performed in rats.

Tucatinib has a low risk of mutagenicity. Tucatinib was non-mutagenic when tested using bacterial reverse mutation (Ames test) or L5178Y/TK+/- mouse lymphoma cell assays. In addition, at doses up to 2000 mg/kg, tucatinib was negative for the induction of micronucleated polychromatic erythrocytes in mice.

Important data on **potential embryo-toxicity of tucatinib were obtained in preclinical studies in pregnant rabbits.** Tucatinib doses of ≥90mg/kg/day lead to fetal external and visceral malformations, including domed head with severe dilation of the lateral and third ventricles. Other malformations included hyperflexed forepaw, herniated umbilicus, organ malposition, and vascular malformations and variations. **Given preclinical findings of embryo-toxicity, all women of childbearing potential should have negative pregnancy test prior to staring tucatinib, and should use effective measures of contraception while on tucatinib and 30 days after stopping tucatinib.**

2.1.5 Pharmacokinetics, absorption, distribution, metabolism and excretion

Pharmacokinetic (PK) studies conducted with tucatinib in mice, rats, and monkeys showed good exposure in all three nonclinical species with calculated absolute oral bioavailability (F) values of 22% to 34% in rats and exceeding 100% in monkeys. Tucatinib is a high permeability compound. It is both an inhibitor and a substrate for P-glycoprotein and is highly, but reversibly, bound to plasma proteins *in vitro* across multiple species (>93% bound). Tucatinib demonstrates good-to-moderate stability with respect to hepatic metabolism across species. Tucatinib is metabolized by CYP450 enzymes in human liver, primarily by CYP2C8 and to a lesser extent by CYP3A4. Strong CYP2C8 and CYP3A4 inhibitors or inducers should not be administered in combination with tucatinib in order to minimize the risk of potential drug-drug interaction. Tucatinib exhibited moderate *in vitro* inhibition of CYPs 2C8 and 2C9 in addition to moderate inhibition of UDP-glucuronosyltransferase 1A1 (UGT1A1). *In vivo* evaluations of [14C]-tucatinib showed tucatinib-related radioactivity distributed to the liver, lungs, and kidneys and excreted predominantly into bile and feces, with ONT-993 being the predominant metabolite.

In vitro, tucatinib inhibited CYP3A4 at concentrations approximately 10 times higher than clinically relevant concentrations; time-dependent inhibition of CYP3A4 was not observed and tucatinib did not induce *in vitro* activity of CYP3A4 or CYP1A2 in human hepatocytes. However, different results in respect to CYP3A4 inhibition were obtained in healthy volunteers. Preliminary pharmacokinetic (PK) results from Study ONT-380-012 indicate that coadministration of multiple doses of tucatinib (300 mg BID) with midazolam (a sensitive CYP3A substrate) increased the geometric mean midazolam exposure (AUC) approximately 5.85-fold (90% CI 5.14 to 6.66) in healthy subjects, compared with administration of midazolam alone. **Therefore, tucatinib is a strong CYP3A4 inhibitor, and its use together with sensitive CYP3A4 substrate should be avoided, or doses of sensitive CYP3A4 substrates should be adjusted accordingly.**

2.1.6 Summary of clinical studies

Tucatinib (ARRAY-380, ONT-380) has been investigated in multiple clinical studies. Three studies have been completed, including two Phase 1 formulation studies in healthy subjects and a Phase 1 single-agent, dose-escalation study with an expansion cohort in HER2+ breast cancer patients at the MTD (Table 1). Other clinical trials are underway to examine the safety and efficacy of tucatinib when combined with other anti-HER2 therapies (Table 2). The most important selected clinical studies are highlighted below in sections 2.1.7 – 2.1.9.

Table 1. Completed Clinical Studies

Study ID	Design/Objective	Treatment	Population/ Enrollment	Results
ARRAY-380-101	Phase 1, single agent, open-label, dose-escalation study to identify the MTD/RP2D of Array-380 capsules (PIC) and to assess safety, PK, and preliminary efficacy.	Dose-escalation phase: 25 to 800 mg PO BID in 28-day cycles. Expansion phase: 600 mg PO BID in 28-day cycles	HER2+ metastatic breast cancer. 50 subjects enrolled, including 31 at doses ≥ 600mg	MTD 600 mg PO BID (PIC). Among 35 subjects evaluable for efficacy, 5 subjects with PR (14%), 18 subjects with SD (51%)

ARRAY-380-102	Phase 1, open-label, single-dose, fixed-sequence, 4-period crossover study to evaluate the PK, relative bioavailability, and safety of four Array-380 formulations in healthy subjects	Single 300 mg PO dose of Array-380 in 4 treatment formulations: 1. Capsules (PIC) 2. Micronized PIC 3. Aqueous suspension 4. Captisol®/apple juice solution	14 healthy subjects	The relative bioavailability of ARRY-380 (AUC and C_{max}) was higher following the micronized PIC and aqueous suspension formulations, and lower following the 20% Captisol®/apple juice solution compared to the control capsule (PIC formulation)
ARRAY-380-103	Phase 1, open-label, single-dose, fixed-sequence, 4-period crossover study to evaluate the PK, relative bioavailability, potential food effect, omeprazole drug interaction, and safety of Array-380 PO capsules and tablets in healthy subjects	Single 300 mg PO dose of Array-380 in each of 4 treatment periods: 1. Capsules (PIC) 2. Tablets (fasted) 3. Tablets (fed) 4. Tablets (fasted) following omeprazole 40 mg x 5 days	12 healthy subjects	Array-380 metabolism was not affected by formulation, food, or gastric pH. Tablet and capsule had similar bioavailability. Higher drug exposure with the tablet formulation in fed state
ONT-380-008	Phase I single-dose, open-label study to (1) Determine the mass balance of total radioactivity from [14C]-tucatinib (2) Determine the routes and rates of elimination of total radioactivity from [14C]-tucatinib (3) Determine the urinary excretion of tucatinib and the metabolite ONT-993 following administration of [14C]-tucatinib (4) Characterize the PK of tucatinib, ONT-993, and total radioactivity following administration of [14C]-tucatinib to healthy male and female subjects	Single dose of 300 mg of [14C]-tucatinib containing approximately 150 µCi of [14C] radioactivity, administered as an oral solution after an overnight fast.	8 healthy subjects	Determined routes and rates of elimination of tucatinib and its metabolite ONT-993 and characterized their PKs
ONT-380-009	Phase I single-dose, open-label study to evaluate the PK profile	Tucatinib 300mg as a single dose	37 hepatically impaired and	No difference in plasma exposure of tucatinib between

	of tucatinib in subjects with impaired hepatic function compared to control subjects		healthy subjects	subjects with normal hepatic function and with mild hepatic impairment, however, in subjects with moderate or severe impairment tucatinib exposure is increased
ONT-380-011	Phase I single-dose, open-label study to evaluate the PK profile of tucatinib in subjects with impaired hepatic function compared to control subjects	Treatment A: tucatinib 300 mg PO Treatment B: placebo PO Treatment C: moxifloxain 400 mg PO	53 healthy subjects	No changes in cardiac repolarization on tucatinib
ONT-380-012	Phase I open-label, fixed-sequence, 5-part study: Part A: to assess the effect of a strong CYP3A4 inhibitor (itraconazole) on the single dose PK of tucatinib. Part B: to assess the effect of an inducer of CYP3A4 and CYP2C8 (rifampin) on the single dose PK of tucatinib. Part C: to assess the effect of a strong CYP2C8 inhibitor (gemfibrozil) on the single dose PK of tucatinib. Part D: to assess the effects of tucatinib on the single dose PK of substrate probes of CYP2C8 (repaglinide), CYP2C9 (tolbutamide), and CYP3A4 (midazolam). Part E: to assess the effect of tucatinib on the single dose PK of a substrate probe of P-gp (digoxin).	Tucatinib 300 mg PO and itraconazole 200 mg (Part A), rifampin 600 mg (Part B), gemfibrozil 600 mg (Part C), repaglinide 0.5 mg (Part D), tolbutamide 500 mg (Part D), midazolam 500 mg (Part D), and digoxin 5 mg (Part E)	116 healthy subjects	Data on tucatinib metabolism and drug-drug interaction
SGNTUC-015	Phase I open-label study to evaluate the PK of tucatinib and its	Tucatinib 50, 150, 300 mg PO	36 healthy subjects	PKs of tucatinib in Asian patient population

	metabolite (ONT-993) in Japanese and Caucasian subjects			
SGNTUC-020	Phase I open-label study to assess the effects of multiple BID oral doses of tucatinib on the single-dose PK of metformin, a substrate of MATE1/2-K.	Tucatinib 300 mg PO Metformin 850 mg PO Iohexol 1500 mg IV	18 heatlhy subjects	Data on tucatinib metabolism and drug-drug interaction

Abbreviations: twice daily (BID); immunohistochemistry (IHC); fluorescence in situ hybridization (FISH); maximum-tolerated dose (MTD); oral (PO); powder in capsule (PIC); pharmacokinetics (PK); area under the curve (AUC); C_{max} (maximum concentration observed); partial response (PR); recommended Phase 2 dose (RP2D); stable disease (SD).

Table 2. Ongoing Clinical Studies

Study ID	Design/Objective	Treatment	Status
ONT-380-004	Phase 1b, open-label, dose-escalation trial to determine the MTD/RP2D of ONT-380 tablets given in combination with T-DM1 in patients with metastatic breast cancer previously treated with trastuzumab and a taxane. Expansion cohort in subjects with CNS metastasis at the MTD/RP2D. (Sponsor: Oncothyreon – now Seattle Genetics, Inc.)	**Dose-escalation**: Tucatinib: 300 mg or 350 mg PO BID T-DM1: 3.6 mg/kg IV on Day 1 21-day cycles **Expansion (at MTD)**: Tucatinib: 300 mg PO BID T-DM1: 3.6 mg/kg IV on Day 1 21-day cycles	Active, completed enrollment of 57 subjects. MTD of ONT-380 in combination with T-DM1 is 300 mg PO BID
ONT-380-005	Phase 1b, open-label, dose-escalation trial to determine the MTD/RP2D of ONT-380 tablets given in combination with trastuzumab, capecitabine, and trastuzumab + capecitabine in patients with metastatic breast cancer previously treated with trastuzumab and T-DM1. Expansion cohort in patients with CNS metastasis at the MTD/RP2D (Sponsor: Oncothyreon – now Seattle Genetics, Inc.)	ONT-380 in escalating doses, starting at 300 mg PO BID. Capecitabine at 1000 mg/m2 PO BID on Days 1–14 of each 21-day cycle. Trastuzumab as a loading dose of 8 mg/kg followed by 6 mg/kg once q21 days	Active, completed enrollment. RP2D of ONT-380 in combination with capecitabine, trastuzumab, and capecitabine + trastuzumab is 300 mg PO BID
ONT-380-206 (HER2CLIMB) NCT02614794	Phase II randomized double-blinded controlled study of ONT-380 (tucatinib) vs placebo in combo with capecitabine and trastuzumab	Tucatinib 300mg PO BID or placebo in combination with capecitabine 1000 mg/m2 PO BID on	Ongoing

	in subjects with metastatic HER2+ breast cancer (Sponsor: Oncothyreon – now Seattle Genetics, Inc.)	days 1-14 of each 21-day cycle, and with trastuzumab 6 mg/kg IV every 21 days	
HER2CLIMB-02 (SGNTUC-016)	Phase III randomized, double-blind, placebo-controlled to compare PFS by investigator assessment per RECIST v1.1 between treatment arms	Tucatinib or Placebo: 300 mg PO BID T-DM1: 3.6 mg/kg IV every 21 days	Ongoing
MOUNTAINEER (SGNTUC-017)	Phase II randomized, open-label to determine the antitumor activity of tucatinib given in combination with trastuzumab, in Cohorts A+B, as measured by confirmed ORR per RECIST 1.1, according to BICR assessment	Tucatinib 300 mg PO BID Trastuzumab: 8 mg/kg IV Day 1 Cycle 1, 6 mg/kg IV Day 1 subsequent cycles 21-day cycles Cohorts A+B = tucatinib + trastuzumab Cohort C = tucatinib monotherapy	Ongoing
380-IST-001 DFCI 13-198	Phase 1, dose-escalation trial of ARRAY-380 (aka ONT-380) tablets in combination with trastuzumab in participants with brain metastases from HER2+ breast cancer to determine MTD/RP2D of ONT-380 given in combination with trastuzumab (Sponsor: Dana Farber Cancer Institute)	Array/ONT-380 in escalating doses, starting at 450 mg PO BID, given irrespective of food. Additional arm with once daily dosing of ONT-380 starting at 750 mg PO daily. Trastuzumab at 6 mg/kg IV q3 weeks	Ongoing
380-IST-002 Academic and Community Cancer Research United	A Phase II, Open Label Study of Tucatinib Combined With Trastuzumab in Patients With HER2+ Metastatic Colorectal Cancer (MOUNTAINEER)	Tucatinib (tablet formulation) 300 mg PO BID of every 21-day cycle and trastuzumab 8 mg/kg IV loading dose Day 1 Cycle 1, followed by 6 mg/kg IV Day 1 of all subsequent cycles	Ongoing

Abbreviations: twice daily (BID); central nervous system (CNS); intravenous (IV); maximum-tolerated dose/recommended Phase 2 dose (MTD/RP2D); orally (PO).

2.1.7 Clinical study ARRAY-380-101

Array-380 has been studied in an open-label, single-agent, Phase 1 dose-escalation study with an MTD expansion cohort conducted at four sites in the US and Canada. Array-380 was administered in capsule (PIC) on a BID dosing schedule. The study was originally designed to enroll subjects with advanced solid tumors known to express HER2. Later, it was amended to allow only subjects with documented HER2+ cancers. This study was designed to identify the MTD and to assess the safety, PK, and preliminary efficacy of Array-380 capsules with doses

ranging from 25 to 800 mg administered PO BID. A total of 33 patients were treated in the dose-escalation phase, and 17 additional patients with HER2+ metastatic breast cancer in the expansion cohort. Patients were heavily pretreated, with a median number of five prior treatment regimens. All breast cancer patients had received prior trastuzumab, and 88% of these patients had received prior lapatinib. Treatment-emergent adverse events (TEAEs) occurring in ≥ 20% of patients, regardless of relationship to study drug, were nausea (56%); diarrhea (52%); fatigue (48%); vomiting (40%); rash (24%), constipation, cough, and pain in extremity (20% each). Of these events, the majority were Grade 1 in severity. Grade 3 TEAEs were reported by 21 of 50 patients (42%) and those occurring in > 1 patient included anemia and cellulitis (6% each); and abdominal pain, hypokalemia, increased alanine aminotransferase (ALT), increased aspartate aminotransferase (AST), musculoskeletal chest pain, and vomiting (4% each). In patients treated at the MTD, Grade 3 events included 1 event each of diarrhea (of 1 day duration), erythematous rash, and ALT/AST elevation. The 800 mg BID dose was deemed a non-tolerated dose due to the occurrence of dose-limiting toxicities (DLT) in 2 of 4 patients treated at that level, both of whom experienced Grade 3 liver transaminase elevation, which reversed upon interruption of therapy. Both patients were able to resume treatment at the next lower dose level. 600 mg BID was declared to be the single-agent MTD for the PIC formulation. Seventeen additional patients were enrolled in the expansion phase of the study at the MTD. Among the 35 patients evaluable for efficacy at any dose level, best response per Response Evaluation Criteria in Solid Tumors (RECIST) was PR in 5 patients (14%), SD in 18 patients (51%), and progressive disease (PD) in 12 patients (34%). Among patients treated at doses of ≥ 600 mg BID with measurable disease at baseline, the CBR was 27% (6/22), including 3 PRs and 3 SDs. All 3 patients with PR had received prior trastuzumab, and 2 had also received prior lapatinib.

2.1.8 Clinical study ONT-380-004

ONT-380 was evaluated in a Phase 1b dose-escalation study in combination with TDM-1 in subjects with metastatic HER2+ breast cancer previously treated with both trastuzumab and a taxane. This study utilized the tablet formulation of ONT-380 rather than the PIC formulation used in ARRAY-380-101. The purpose of this study was to identify the MTD/RP2D of ONT-380 administered BID in combination with the approved dose of T-DM1 of 3.6 mg/kg every 21 days. Other objectives include evaluation of safety, PK, and preliminary efficacy. Three initial dose-escalation cohorts of ONT-380 were planned: 300 mg BID, 450 mg BID, and 600 mg BID. Patients with untreated CNS metastases or treated stable CNS metastases were eligible for the initial dose-escalation cohorts and MTD/RP2D expansion cohort. Patients with either untreated CNS metastases or CNS metastases that had progressed after previous radiation were eligible for the CNS expansion cohort. The study is active with completed enrollment. The first patient treated in the 300 mg cohort experienced a DLT of reversible Grade 3 AST/ALT elevation. The cohort was subsequently expanded and enrolled 7 additional patients, with no additional DLTs. However, PK results from this group suggested that ONT-380 drug exposure at the 300 mg BID dose (tablet formulation) was similar to that observed at the single-agent MTD dose of 600 mg PO BID using the PIC formulation. As a result, the ONT-380 300 mg BID cohort was further expanded to include 10 additional patients to allow better characterization of ONT-380 PK and safety. Two additional patients experienced DLT in the expanded 300 mg cohort: one case of reversible Grade 3 ALT/AST elevation, and one case of Grade 2 vomiting with complicating features (Grade 1 diarrhea), for an overall DLT rate of 3/18 (17%). A 50 mg tablet became available, and a new cohort was opened using ONT-380 at 350 mg BID with the same standard dose of T-DM1. At this

dose, 3/7 pts (43%) experienced DLT (one case each of Grade 3 vomiting, Grade 3 hypersensitivity, and Grade 3 fatigue). This dose was therefore found to be not tolerated, and the MTD for ONT-380 for this combination was declared to be 300 mg PO BID. The protocol was subsequently amended to allow up to 30 patients to be treated at the MTD/RD expansion cohort to further characterize the safety and PK of the combination. The most common AEs (occurring in >10% of patients), regardless of relationship, have been nausea, fatigue, diarrhea, vomiting, thrombocytopenia, AST/ALT elevation, headache, decreased appetite, constipation, and hypokalemia. Grade 3 AST/ALT elevation has occurred in 7/43 pts (16%), with no events meeting Hy's law (AST/ALT >3X upper limit of normal (ULN) with elevation of serum total bilirubin to ≥2X ULN without initial findings of cholestasis and no other explanation of liver findings) [60] and has been reversible with dose interruption and reduction except in 2 patients found to have progressive liver metastases. ONT-380 PK has been dose proportional, and no drug-drug interaction has been observed with T-DM1. In 33 of 43 subjects with data available from at least one follow-up scan to evaluate response, best systemic response regardless of dose was 11 PR, 16 SD, and 6 PD, with CBR of 19/33 (58%). The most common reason for treatment discontinuation was PD, with 3 subjects coming off study for AEs (n=1 each of Grade 3 hypersensitivity, Grade 2 vomiting, Grade 3 AST/ALT elevation). In conclusion, in this study, tucatinib in combination with T-DM1 appeared to have acceptable toxicity and to show preliminary antitumor activity among heavily pretreated patients with ERBB2/HER2-positive metastatic breast cancer with and without brain metastases [61].

2.1.9 Clinical study ONT-380-005

ONT-380 in a tablet formulation has been evaluated in a Phase 1b dose-escalation study with trastuzumab and capecitabine in patients with metastatic HER2+ breast cancer previously treated with both trastuzumab and T-DM1. It is a parallel dose-escalation study to evaluate the MTD/RP2D of ONT-380 in combination with either trastuzumab (8 mg/kg followed by 6 mg/kg once q21 days) or capecitabine (1000 mg/m2 PO BID on Days 1–14 of each 21-day cycle), followed by evaluation of the MTD/RP2D for the three-drug combination. Other objectives include assessment of safety, PKs, and anti-tumor activity of the three different combinations. Three initial dose-escalation cohorts of ONT-380 were planned: 300 mg BID, 450 mg BID, and 600 mg BID. Patients with untreated CNS metastases or treated stable CNS metastases were eligible for the initial dose escalation cohorts and MTD/RD expansion cohort. Patients with either untreated CNS metastases or CNS metastases that had progressed after previous radiation were eligible for the CNS expansion cohorts. Two cohorts were initially opened, using ONT-380 at a dose of 300 mg BID combined with either capecitabine or trastuzumab. No DLTs were seen in either of these initial two cohorts, and enrollment was expanded to better characterize the safety and PK of these two drug combinations, with 7 patients treated with ONT-380 300 mg BID plus capecitabine, and 9 patients treated with ONT-380 300 mg BID plus trastuzumab. Initial PK data from these cohorts along with data from the concurrent ONT-380-004 study suggested that ONT-380 drug exposure at the 300 mg BID dose level was similar to that seen with the single agent MTD dose of 600 mg PO BID using the PIC formulation. Therefore, the study safety monitoring committee recommended against dose escalation to the original planned 450 mg BID dose level, and instead the three drug combination cohort of ONT-380 + capecitabine and trastuzumab was opened using ONT-380 at a dose of 300 mg BID. One of the first three patients treated in this cohort experienced DLT (grade 4 cerebral edema confirmed by MRI). This patient had an untreated metastasis in the thalamus and had neurologic symptoms on Cycle 1 Day 7. The patient's

symptoms resolved within 48 hours after initiating treatment with corticosteroids. She discontinued capecitabine permanently and held ONT-380 for two weeks, with no recurrence of symptoms upon ONT-380 rechallenge. The cohort was subsequently expanded per dose escalation guidelines, and no additional DLTs have been seen in the other 10 patients in this cohort. Following the availability of 50 mg tablets, two new dose escalation cohorts were opened evaluating ONT-380 at 350 mg BID in combination with either capecitabine alone (n=4) or trastuzumab alone (n=4). While no events occurred during the protocol-defined period to qualify for a DLT for either of these combinations at this dose, two patients experienced events leading to dose reductions or discontinuations of ONT-380 in later cycles due to GI toxicity, including one case each of Grade 3 diarrhea and Grade 3 nausea/vomiting. Because of these events, along with PK data showing that patients treated at 350 mg BID of the tablet formulation had drug exposure above that of patients treated at the single agent MTD of the PIC formulation, the study safety monitoring committee declared RP2D as 300 mg BID for all combinations in the study, and MTD/RP2D non-CNS and CNS expansion cohorts were opened for both the trastuzumab and capecitabine/trastuzumab combinations. The study is active, completed enrollment. Adverse events seen at the recommended phase 2 dose regardless of causality, grade, and treatment group included diarrhea (35 [67%] of 52 patients), nausea (31 [60%] patients), palmar-plantar erythrodysesthesia syndrome (23 [44%] patients), fatigue (20 [38%] patients), and vomiting (20 [38%] patients). In all patients, treatment-related toxicities of grade 3 and worse included fatigue (five [8%] patients), diarrhea (four [7%] patients), and palmar-plantar erythrodysesthesia (four [7%] patients). No treatment-related deaths were reported. The proportion of patients with measurable disease achieving objective response was 83% (five of six patients) in the combination of tucatinib with capecitabine, 40% (six of 15 patients) in the combination of tucatinib with trastuzumab, and 61% (14 of 23 patients) in the combination of tucatinib with both capecitabine and trastuzumab. In conclusion, tucatinib in combination with capecitabine and trastuzumab had acceptable toxicity and showed preliminary anti-tumor activity [62].

2.1.10 Clinical pharmacokinetic results

Pharmacokinetics parameters were determined in a Phase 1 single dose range finding study with MTD expansion cohort (ARRAY-380-101) using a PIC formulation. Dose-proportionality was observed across the dose range, ARRAY-380 time of maximum concentration observed (Tmax) ranged from 1 hr to 4 hrs with elimination half-life (t1/2) of 5 to 7 hrs. Steady state pharmacokinetics from the 600 mg BID MTD showed a mean drug exposure, the daily area-under-the-curve (AUC daily), of 10020 h.ng/ml with a mean C_{max} of 835 ng/ml, and a median t1/2 of 5 hours. When dosed at the MTD, ARRAY-380 levels were maintained at or above the predicted IC_{90} at steady state. A tablet formulation was developed for ONT-380 and the pharmacokinetics of the tablet formulation was compared to the PIC capsule formulation. The tablet resulted in lower inter-subject variability compared to the capsule. Food increased exposure of ONT-380/tucatinib from the tablet by approximately 50% after administration of the tablet in the fed state compared to fasted state. Absorption of tucatinib from the tablet was delayed when administered with food, but C_{max} was not affected. The tablet formulation of tucatinib was subsequently used in the studies tucatinib-004 and tucatinib-005. PK results from these two studies indicated that the tablet formulation resulted in a nearly 2-fold increase in drug exposure at steady state when compared to the PIC formulation. At steady state and at the 300 mg BID dose, the mean C_{max} of tucatinib was 802 ng/ml, the mean time to C_{max} was 2hrs, the mean half-life was 7 hrs, and the mean AUC daily was 10207 h.ng/ml, almost exactly the same as the value of the AUC daily obtained from the 600

mg BID MTD of PIC formulation. The PKs of tucatinib tablets were consistent and proportional over the doses studied when given either as a single agent or in combination with other agents. There was no unusual drug-drug interactions observed between tucatinib and the combined drugs capecitabine and trastuzumab. PK analysis of capecitabine, TDM-1 and their catabolites/metabolites showed plasma levels that were consistent with the published data.

2.1.11 ONT-380 / tucatinib in the treatment of CNS metastases

ONT-380-004 (tucatinib + TDM-1) and ONT-380-005 (tucatinib + capecitabine, trastuzumab or capecitabine and trastuzumab) studies have enrolled subjects with previously treated stable CNS metastases, untreated CNS metastases, or previously treated and progressive CNS metastases. Patients were considered evaluable for CNS response if they had CNS lesions that had never been treated, or lesions that had progressed after prior local therapy (either WBRT, SRS, or surgical resection). Patients were considered not evaluable for CNS response if they had CNS metastases which were previously treated with local therapy and were stable or decreased in size since local therapy. CNS response was evaluated using a modified RECIST 1.1 criteria, as well as considerations for neurological deterioration and steroid use. Seventeen subjects from these two studies had evaluable CNS lesions at baseline (tucatinib plus TDM-1 n=9; tucatinib plus capecitabine n=1; tucatinib plus trastuzumab n=4; and tucatinib plus capecitabine and trastuzumab n=3). Of these, 14 underwent at least one follow-up CNS assessment and were evaluable for CNS response. In these 14 subjects, best overall CNS response was 1 CNS complete response (CR) (T-DM1 n=1), 4 CNS PR (TDM-1, n=2; capecitabine n=1, capecitabine plus trastuzumab n=1), and 9 CNS SD (TDM-1, n=5; trastuzumab, n=3; capecitabine plus trastuzumab, n=1). Responses in the CNS were seen even in subjects treated previously with pertuzumab, TDM-1, and lapatinib. Of the three subjects who were non-evaluable for CNS response, 2 had systemic PD and no follow-up CNS scan (TDM-1, n=1; capecitabine plus trastuzumab, n=1), and 1 subject treated with tucatinib and trastuzumab alone with an enlarging lesion was taken off study for surgical resection. Pathologic review of the resected specimen revealed no viable tumor and evidence for treatment-induced necrosis. Four subjects (TDM-1, n=2; trastuzumab, n=1; capecitabine, n=1) were on treatment for > 6 months. In summary, tucatinib has demonstrated early signs of activity in CNS, resulting in radiographic tumor responses as well as prolonged stabilization of disease.

2.2 Palbociclib

2.2.1 Mechanism of action

Palbociclib is an inhibitor of CDK4/6 complex. CDK4 and CDK6 regulate cycle progression from G1 into S phase. Major proteins in the CDK4/6 pathway are CDK4, CDK6, their partner cyclin D1, and tumor suppressors P16 and **r**etino**b**lastoma (Rb) protein.

Approximately 15% of human breast cancers demonstrate amplification of the cyclin D1 gene, *CCND1*, and the majority of human breast cancers show overexpression of this protein [63, 64]. In regulation of cell cycle progression, cyclin D1 interacts closely with the Rb protein. In the hypo-phosphorylated state, Rb acts as a tumor suppressor and contributes to cell cycle regulation at the G1 to S checkpoint by causing G1 arrest and suppressing gene transcription that is required for entry into the S phase. In response to mitogenic signals, CDK4 and CDK6 form a complex with cyclin D1, which phosphorylates the Rb protein. Once phosphorylated, phospho-RB disassociates from the transcription factor E2F1, liberating E2F1 from its cytoplasm bound state and allowing it to enter the nucleus, where it promotes the transcription of target genes that are essential for transition from G1 to S phase [65]. Controlled phosphorylation and deactivation of

the Rb protein by the CDK4/CDK6/cyclin D1 complex is essential for progression of the cell cycle from G1 to S phase, and for cellular proliferation [64]. Loss of function of phospho-Rb has been described in 20 to 35% of breast cancers [66].

Inhibition of cyclin D1 via inactivation or degradation leads to cell cycle exit and differentiation. Inactivation of cyclin D1 is triggered by an inhibitor protein P16 which bind to CDK4/CDK6/cyclin D1 complex and inactivates the whole complex [65]. P16 gene deletions and/or promoter hypermethylation are frequent in breast carcinomas [67, 68]. CDK4/6 and cyclin D1 receive upstream regulation via ER, MAP kinase, β-catenin and PI3K pathways. The MAP kinase ERK activates the downstream transcription factors Myc and AP-1, which, in turn, activate the transcription of the CDK4, CDK6 and cyclin D1 genes. Rho family GTPases and focal adhesion kinase activate cyclin D1 gene in response to integrins [69, 70]. Importantly, there is a cross-talk between estrogen receptor α (ERα) and cyclin D1 pathways in HR+ breast cancer: 17β-estradiol bound to ERα induces cyclin D1 gene expression, while cyclin D1 directly binds to ERα and stimulates ligand-independent ER-signaling [71]. Unrestricted CDK4/6 pathway activity, typical of HR+ breast cancer, leads to growth advantage and uncontrolled cell proliferation. In vitro, palbociclib reduces proliferation of HR+ breast cancer cell lines by blocking progression of the cell from G1 into S phase of the cell cycle [14]. Treatment of HR+ breast cancer cell lines (regardless of the HER2 status) with the combination of palbociclib and antiestrogens leads to decreased Rb phosphorylation resulting in a reduced expression of E2F1 transcription factor, involved in the cell cycle regulation and synthesis of DNA. This leads to cell cycle arrest and increased growth inhibition compared to treatment with each drug alone [72]. *In vitro* treatment of HR+ breast cancer cell lines with the combination of palbociclib and antiestrogens causes increased cell senescence sustained for several days following drug removal [72, 73]. *In vivo* studies using a patient-derived breast cancer xenograft model demonstrated that the combination of palbociclib and letrozole increased Rb phosphorylation, and suppressed CDK4/6 downstream signaling and tumor growth to a greater extend compared to palbociclib or letrozole alone [72].

2.2.2 Pharmacokinetics: absorption, distribution, metabolism and excretion

Absorption: The mean C_{max} of palbociclib is observed between 6 to 12 hours following oral administration. The mean absolute bioavailability of palbociclib after an oral 125 mg dose is 46%. In the dosing range of 25 mg to 225 mg, the AUC and C_{max} increase proportionally with the dose. Steady state is achieved within 8 days following repeated once daily dosing. Palbociclib absorption and exposure are very low in approximately 13% of the population under the fasted condition. Food intake increases the palbociclib exposure in this small subset of the population, but does not alter palbociclib exposure in the rest of the population to a clinically relevant extent. Therefore, food intake reduces the intersubject variability of palbociclib exposure, which supports administration of palbociclib with food.

Distribution Binding of palbociclib to human plasma proteins in vitro is approximately 85%, with no concentration dependence over the concentration range of 500 ng/mL to 5000 ng/ml. The geometric mean apparent volume of distribution is 2583 L (26% CV).

Metabolism: Palbociclib undergoes hepatic metabolism via oxidation and sulfonation, with acylation and glucuronidation contributing as minor pathways. Palbociclib is the major circulating drug-derived entity in plasma (23%). The major circulating metabolite is a glucuronide conjugate of palbociclib, although it only represents 1.5% of the administered dose in the excreta. Palbociclib is extensively metabolized with unchanged drug accounting for 2.3% and 6.9% of

excreted dose in feces and urine, respectively. In feces, the sulfamic acid conjugate of palbociclib is the major drug-related component, accounting for 26% of the administered dose. Palbociclib is primarily metabolized by sulfotransferase (SULT) enzyme SULT2A1 and CYP3A.

Elimination: The geometric mean apparent oral clearance of palbociclib is 63.1 L/hr (29% CV), and the mean (± standard deviation) plasma elimination half-life is 29 (±5) hours in patients with advanced breast cancer. In 6 healthy male subjects given a single oral dose of palbociclib, a median of 91.6% of the total administered dose was excreted in 15 days; feces (74.1% of dose) was the major route of excretion, with 17.5% of the dose recovered in urine. The majority of the material was excreted as metabolites.

Drug Interactions: Palbociclib is primarily metabolized by CYP3A, and it is a weak time-dependent inhibitor of CYP3A following daily 125 mg dosing to a steady state. Administration of sensitive CYP3A4 substrate midazolam with multiple doses of palbociclib increased midazolam plasma exposure by 61% in healthy subjects, compared with administration of midazolam alone [28]. According to FDA drug interaction labeling (http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#table3-1), this is consistent with palbociclib being a weak CYP3A4 inhibitor. Because of these properties, strong CYP3A inhibitors and inducers should be avoided in combination with palbociclib, or the dose of palbociclib should be modified accordingly. Per FDA prescribing information, dose of palbociclib should be reduced to 75mg daily when combined with strong CYP3A4 inhibitors. The dose of sensitive CYP3A4 substrates with narrow therapeutic indices (listed in Appendix D) may need to be reduced when given concurrently with palbociclib. Palbociclib is not an inhibitor of CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, and 2D6, and is not an inducer of CYP1A2, 2B6, 2C8, and 3A4 at clinically relevant concentrations. Under fed conditions there is no clinically relevant effect of PPIs, H2-receptor antagonists, or local antacids on palbociclib exposure. There is no drug interaction between palbociclib and letrozole when the two drugs are co-administered. Palbociclib has a low potential to inhibit the activities of drug transporters P-glycoprotein, breast cancer resistance protein, organic anion transporters 1 and 3, organic cation transporter 2, and organic anion transporting polypeptides 1B1 and 1B3 at clinically relevant concentrations. The recommended full dose of palbociclib is a 125 mg tablet taken orally once daily with food for 21 consecutive days followed by 7 days off treatment to comprise a complete cycle of 28 days.

2.2.3 Summary of clinical studies

Palbociclib is indicated for the treatment of postmenopausal women with HR+ metastatic breast cancer in combination with anti-hormonal therapy. Palbociclib received accelerated approval by FDA based on the results of PALOMA-1 randomized phase II clinical trial, where it showed marked improvement in median PFS in combination with letrozole versus letrozole alone (26.1 versus 7.5 months) as a front line therapy of metastatic breast cancer [20]. Palbociclib showed remarkable activity in the second line metastatic settings in combination with fulvestrant in PALOMA-3 clinical trial, resulting in more than doubling of median PFS (9.2 months palbociclib with fulvestrant vs 3.8 months placebo with fulvestrant; HR 0.42; P <0.001) [58].

In PALOMA-1 clinical trial [20], dose reduction of palbociclib due to an adverse reaction of any grade occurred in 36% of patients. Permanent discontinuation of treatment due to an adverse reaction occurred in 8% patients receiving palbociclib and letrozole, and in 3% of patients receiving letrozole. Adverse reactions leading to discontinuation of palbociclib and letrozole included neutropenia (6%), asthenia (1%), and fatigue (1%).

The most common adverse reactions (≥10%) of any grade reported in patients in the palbociclib plus letrozole arm were neutropenia, leukopenia, fatigue, anemia, upper respiratory infection, nausea, stomatitis, alopecia, diarrhea, thrombocytopenia, decreased appetite, vomiting, asthenia, peripheral neuropathy, and epistaxis. The most frequently reported serious adverse reactions in patients receiving palbociclib plus letrozole were pulmonary embolism (4%) and diarrhea (2%). Higher frequency of pulmonary embolism in this study was attributed to a time bias (significantly prolonged progression free survival in patients on palbociclib and letrozole comparing to single agent letrozole). Higher frequency of pulmonary embolism was not confirmed in other studies of palbociclib.

One of the most common adverse events (AEs) on palbociclib is decreased neutrophil count. Grade 3 (57%) or 4 (5%) decreased neutrophil counts were reported in patients receiving palbociclib plus letrozole in the PALOMA-1 randomized clinical trial. Grade ≥3 neutropenia was managed by dose reductions and/or dose delay or temporary discontinuation; the rate of permanent discontinuation of palbociclib due to repeated episodes of neutropenia was 6%. Median time to the first episode of any grade neutropenia per laboratory data was 15 days (13-117 days). Median duration of Grade ≥3 neutropenia was 7 days. Febrile neutropenia events have been reported following palbociclib administration, although no cases of febrile neutropenia have been observed in PALOMA-1 [20]. Recommended monitoring include complete blood count (CBC) prior to starting palbociclib, at the beginning of each cycle, on Day 15 of the first two cycles, and as clinically indicated. Infections have been reported at a higher rate in patients treated with palbociclib in PALOMA-1 trial. Grade 3 or 4 infections occurred in 5% of patients treated with palbociclib plus letrozole whereas no patients treated with letrozole alone experienced a Grade 3 or 4 infection [20]. **Based on findings in animals and mechanism of action, palbociclib can cause fetal harm** (pregnancy category – not currently assigned, likely X).

2.3 Letrozole

Letrozole is a nonsteroidal competitive inhibitor of the aromatase enzyme system which binds to the heme group of aromatase, a cytochrome P450 enzyme which catalyzes conversion of androgens to estrogens (specifically, androstenedione to estrone and testosterone to estradiol). This leads to inhibition of the enzyme and a significant reduction in plasma estrogen (estrone, estradiol and estrone sulfate) levels. Letrozole is administered in the dose of 2.5mg PO daily, and following administration, it is rapidly and well absorbed. Its absorption is not affected by food. Letrozole has a volume of distribution of 1.9 L/kg. It weakly binds to plasma proteins. Letrozole is metabolized in the liver via CYP3A4 and 2A6 to an inactive carbinol metabolite. Letrozole is a minor substrate of CYP2A6 and CYP3A4. It is a strong inhibitor of CYP2A6, and a weak inhibitor of CYP2C19. It may decrease the metabolism of CYP2A6 substrates, coadministration with strong CYP2A6 substrates (dexmedetomidine and ifosfamide) is not recommended. Letrozole may increase the serum concentration of methadone, the dose of methadone may need to be adjusted. Clinically, letrozole does not display any significant interaction with palbociclib or HER2-targeted agents.

Letrozole half-life is about 2 days, and time to steady state in plasma is 2 to 6 weeks. It is excreted mostly (90%) in urine (6% as unchanged drug, 75% as glucuronide carbinol metabolite, 9% as unidentified metabolites). Letrozole is FDA approved for treatment of breast cancer in postmenopausal women (adjuvant treatment of HR+ early breast cancer, extended adjuvant treatment of early breast cancer after 5 years of tamoxifen; treatment of advanced breast cancer with disease progression following antiestrogen therapy; first-line treatment of HR+ or HR-

unknown, locally-advanced, or metastatic breast cancer). Letrozole is generally well tolerated. Frequent adverse reactions (noted in >10% of patients) include edema (7% to 18%), headache (4% to 20%), dizziness (3% to 14%), fatigue (8% to 13%), hypercholesterolemia (3% to 52%), hot flashes (6% to 50%), nausea (9% to 17%), weight gain (2% to 13%), constipation (2% to 11%), weakness (4% to 34%), arthralgia (8% to 25%), arthritis (7% to 25%), bone pain (5% to 22%), back pain (5% to 18%), decreased bone mineral density (5% to 15%), bone fracture (10% to 14%), dyspnea (6% to 18%), cough (6% to 13%), and night sweats (15%). **Letrozole is contraindicated in pregnancy (category D).**

3 OBJECTIVES AND ENDPOINTS

3.1 Phase Ib part

3.1.1 Primary objective

- To evaluate safety and tolerability of tucatinib used in combination with palbociclib and letrozole, and to confirm that current RP2D of tucatinib and FDA approved dosing of palbociclib remains the same in the triplet combination

3.1.2 Secondary objectives

- To perform an assessment of pharmacokinetic (PK) properties of tucatinib and palbociclib
- To assess preliminary efficacy of tucatinib used in combination with palbociclib and letrozole

3.1.3 Primary endpoint

- Safety and tolerability of combination therapy as evaluated by standard NCI CTCAE version 4.03

3.1.4 Secondary endpoints

- Tucatinib and palbociclib plasma concentrations measured during the first and second cycle of therapy
- Assessment of progression free survival (PFS) defined as the time from allocation to the first documented disease progression according to RECIST 1.1 [26] (Appendix C), or death due to any cause, whichever occurs first
- For subjects with brain metastases enrolled in the study, assessment of bi-compartmental PFS in the non-CNS and CNS compartments, defined as the time from allocation to the first documented disease progression according to RECIST 1.1 [26] and/or RANO-BM criteria [27] (Appendix C), or death due to any cause, whichever occurs first
- Assessment of tumor response – complete response (CR), partial response (PR), or stable disease (SD) of 6 months or longer in duration – by RECIST 1.1 (for subjects with CNS metastases by RECIST 1.1 and RANO-BM); overall response rate (ORR) defined as proportion of subjects who had CR or PR; clinical benefit rate (CBR) defined as proportion of subjects with CR, PR and SD; duration of response (DOR) defined as the time from enrollment into clinical trial until objective tumor progression or death whichever occurs first.

3.2 Phase II part

3.2.1 Primary objective

- To assess efficacy of tucatinib used in combination with palbociclib and letrozole by PFS

3.2.2 Secondary objectives

- To evaluate efficacy of tucatinib used in combination with palbociclib and letrozole by ORR, CBR and DOR
- To evaluate safety and tolerability of the combination therapy

3.2.3 Primary endpoint

- PFS, defined as the time from allocation to the first documented disease progression according to RECIST 1.1, or death due to any cause, whichever occurs first
- For subjects with brain metastatic disease enrolled in the study, assessment of bi-compartmental PFS in the non-CNS and CNS compartments, defined as the time from allocation to the first documented disease progression according to RECIST 1.1 and/or RANO-BM criteria, or death due to any cause, whichever occurs first

3.2.4 Secondary endpoints

- Assessment of response – CR, PR, or SD of 6 month or longer in duration – according to RECIST 1.1 (for subjects with CNS disease by RECIST 1.1 and/or RANO-BM); assessment of ORR (CR and PR), CBR (CR, PR and SD), and DOR.
- Incidence, nature and severity of all AEs that occur on or after C1D1 of therapy
- Tucatinib and palbociclib plasma concentrations measured during the first and second cycle of therapy at late time points

3.3 Scientific endpoints / correlative studies for phase IB and phase II parts

Exploratory assessment of potential biomarkers of resistance and response measured before treatment, after 1 treatment cycle, and at disease progression:

- Assessment of selective markers by IHC in formalin fixed paraffin embedded (FFPE) tumor blocks – such as cyclin E, CDK2, retinoblastoma protein (RB), p16, HER3, total AKT, phospho-AKT, and others as determined by results or advances in the field.
- Changes in tumor RNA expression profiles obtained by mRNA Next Generation sequencing in fresh frozen biopsy samples
- Transcript alterations as assessed by mRNA Next Generation sequencing in fresh frozen biopsy samples
- Analysis of tumor specific mutations in circulating tumor DNA (ctDNA) by digital PCR, including activating mutations in estrogen receptor 1 (*ESR1*) and *HER2* genes, and selected mutations revealed by mRNA sequencing, as well as analysis of other circulating markers associated with cancer prognosis or outcomes from treatment
- Correlation between tumor PAM50 subtype and efficacy of therapy

4 STUDY DESIGN

4.1 Overview

This is a multicenter, single arm, open-label, run-in phase Ib / roll-over phase II study of tucatinib in combination with letrozole and palbociclib in subjects with HR+/HER2+ locally advanced unresectable or metastatic breast cancer. The study will enroll post-menopausal women; premenopausal women are eligible if on treatment with, or agreeable to mandatory ovarian suppression. Notably, premenopausal women of childbearing potential (defined as pre-menopausal women who are not permanently sterile due to hysterectomy, bilateral oophorectomy, bilateral tubal ligation, or bilateral tubal occlusion) are required to have negative pregnancy tests prior to treatment.

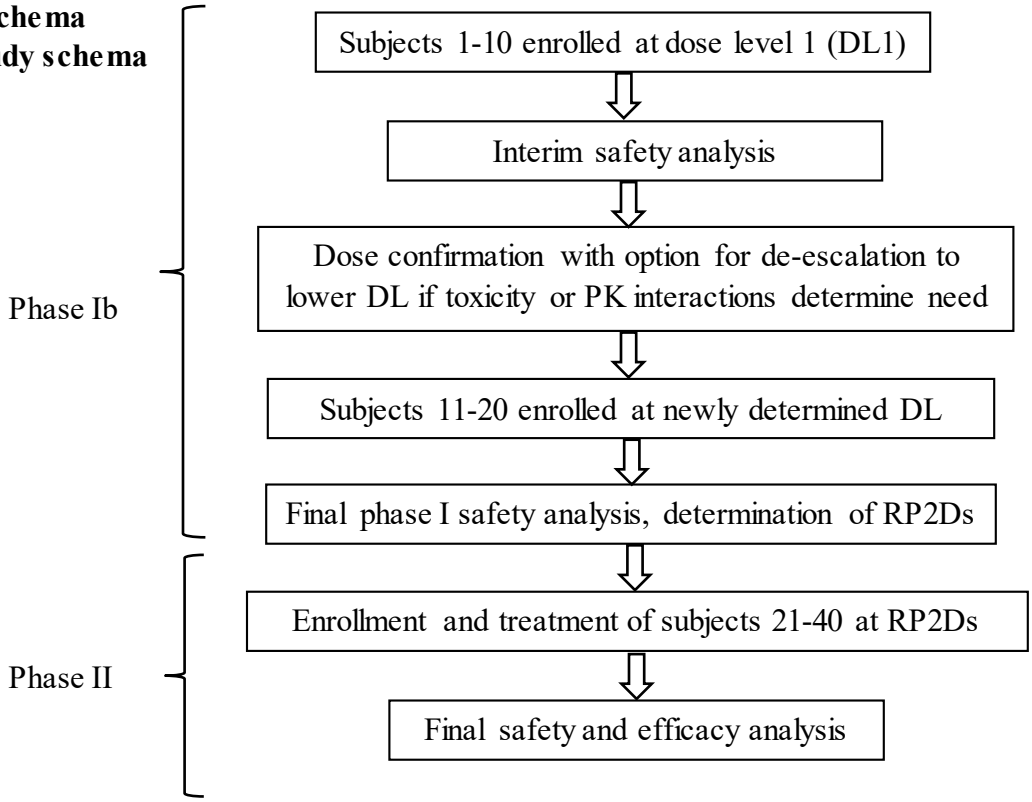
Because of known embryo-toxicity of all three study medications, subjects on treatment will be advised not to become pregnant, and contraception will be required for all women of childbearing potential and their male partners.

The phase Ib part of the study will determine safety and tolerability of the combination of tucatinib, palbociclib and letrozole to confirm that current RP2D of tucatinib and FDA approved dosing of palbociclib remains the same in the triplet combination. The dose of letrozole will be constant through the study period. Once the safety of the combination is established, we will move to the phase II part of the study in the expansion cohort of subjects at RP2D for the purpose of assessing efficacy while further refining assessment of safety of the combination treatment.

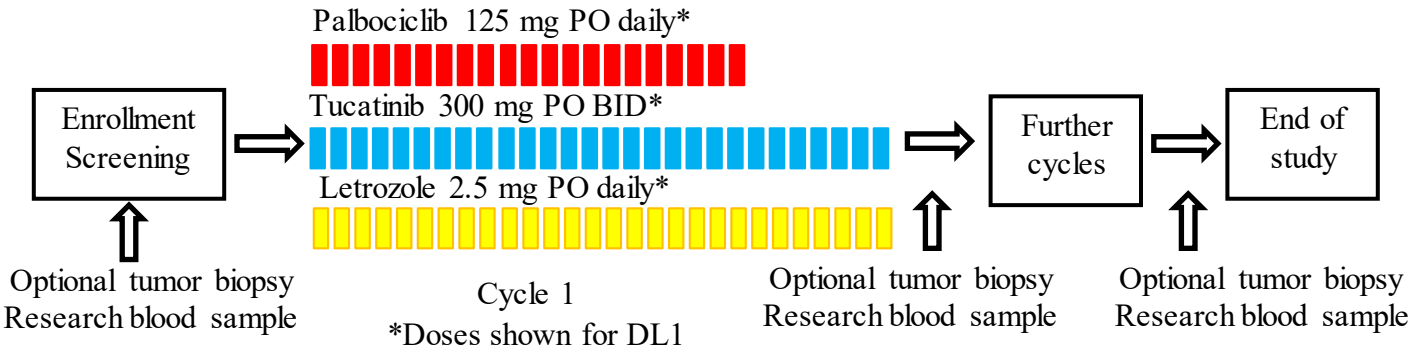
After signing informed consent, completing baseline assessments and meeting all eligibility criteria, the subjects may start treatment. Treatment will be administered in cycles of 28 days each and consist of tucatinib 300 mg PO BID, palbociclib 125 mg PO daily for 21 days followed by 7 days off, and letrozole 2.5 mg PO daily – doses indicated for dose level 1 (DL1). Dose modifications of tucatinib, palbociclib and letrozole will be allowed (as outlined in the section 6.3 of the protocol). Treatment will continue until unacceptable toxicity, disease progression, withdrawal of consent, or study closure. In the absence of clear evidence of clinical or radiographic progression, all efforts should be made to continue treatment until unequivocal evidence of radiologic or clinical progression occurs.

Throughout the study, AEs, SAEs, laboratory values, vital signs, physical examination findings, and ECOG performance status (PS) will be obtained. Toxicity will be evaluated according to NCI-CTCAE, version 4.03. Serial blood samples to measure plasma concentrations of tucatinib and palbociclib will be collected at pre-specified time points as indicated in the Study Calendar table and as described in the section 7.2. The underlying disease status will be assessed by the investigator according to RECIST version 1.1 and RANO-BM criteria (as described in Appendix C) using radiological evaluations.

Fig. 2. Study schema
A: General study schema



B: Sequence of events for a subject on the study:



4.2 Safety analysis of the combination therapy

4.2.1 Dose limiting toxicities

Dose limiting toxicity (DLT) is a side effect attributed to a study drug or drug combination that is serious enough to meaningfully affect the safety of the study subject. Any drug toxicity listed below is a DLT:

- Any death not clearly due to the underlying disease or extraneous causes
- Grade 3 or higher non-hematologic toxicity
- Symptomatic CHF
- Liver toxicity meeting Hy's law
- Grade 3 neutropenia with fever
- Grade 3 thrombocytopenia with bleeding
- Any grade 4 anemia, neutropenia or thrombocytopenia

All AEs of the specified grades should count as DLTs except those that are clearly and incontrovertibly due to disease progression or extraneous causes. Repeated episodes of grade 3 neutropenia interfering with drug administration and necessitating dose reduction of study medications, or other serious toxicity that, in the opinion of investigator merits dose reduction of the study drugs or discontinuation of palbociclib or letrozole according to good clinical practice, will qualify for DLT.

The following adverse events do not represent DLTs:

- Alopecia of any grade
- Grade 3 nausea/vomiting or diarrhea WITHOUT adequate use of antiemetic or anti-diarrheals that improves with optimal medical management in <72hrs
- Grade 3 fatigue lasting ≤ 7 days
- Grade 3 rash WITHOUT optimal use of topical corticosteroids or anti-infectives that improves with medical management in <72 hrs
- ≥ Grade 3 electrolyte abnormality that lasts < 72 hours that is not clinically complicated, and returns to Grade ≤1 spontaneously or after conventional medical interventions
- ≥ Grade 3 amylase or lipase elevation that is not associated with symptoms or clinical manifestations of pancreatitis

In this study, proportion of patients experiencing DLTs will be analyzed during the first and second interim safety analyses. Attribution of the DLTs to specific study drug(s) will be performed as described in section 6.2. Proportion of patients that experienced DLTs prior to the first interim safety analysis will determine if the study meets pre-specified safety thresholds, and whether de-escalation of the study drug doses to lower dose levels is needed. Analysis of DLTs experienced by all patients enrolled in phase I part of the study will determine the outcomes of the second interim safety analysis, and whether the combination of tucatinib, letrozole and palbociclib demonstrates adequate safety profile allowing to proceed to phase II.

4.2.2 Safety analysis algorithm

Incidence, nature and severity of all AEs that occur on therapy will be analyzed. As a signal of lack of safety and tolerability of combination therapy, we will look into proportion of subjects experiencing DLTs due to tucatinib and palbociclib. The known frequency of DLTs for palbociclib as standard of care therapy when administered in combination with letrozole in the current FDA approved setting is significantly higher than the rate of DLTs experienced with tucatinib to date. Therefore, the phase Ib part of the study will be structured to identify an overall clinically meaningful increase in the expected toxicity of palbociclib and/or tucatinib as follows: safety will be considered clinically meaningfully altered if 60% or greater proportion of subjects experience DLTs secondary to the toxicity of palbociclib, or 20% or greater proportion of subjects experience DLTs secondary to the toxicity of tucatinib, or 50% or greater proportion of subjects experience DLTs that can be potentially attributed to both drugs (Tables 3 and 4).

Table 3. Baseline and expected toxicities of palbociclib and tucatinib

Toxicity attributable to a drug	Baseline rate of DLTs with single agent therapy	Rate of DLTs triggering safety concern of combination therapy trial
palbociclib	45% [20]	≥60%
tucatinib	10% [22]	≥20%
toxicity potentially attributable to both tucatinib and palbociclib		≥50%

Table 4. Safety boundaries for phase Ib part of the trial

Drug	Confidence level	N*	Rate	Upper limit CI	n**
palbociclib	0.9	10	0.6	0.812	8 out of 10
		20	0.6	0.751	15 out of 20
tucatinib	0.9	10	0.2	0.408	4 out of 10
		20	0.2	0.347	7 out of 20
both drugs	0.9	10	0.5	0.760	8 out of 10
		20	0.5	0.694	14 out of 20

*N – total number of subjects; **n – number of subjects experiencing DLTs due to palbociclib and/or tucatinib that is set as a safety boundary

Table 5. Pre-planned dose levels (DLs) for subjects in phase Ib part of the study

DLs	Drug doses	Subjects treated
DL1	tucatinib 300 mg PO BID palbociclib 125 mg PO daily 21 day on, 7 days off letrozole 2.5 mg PO daily	Subjects 1-10 treated at DL1
DL -1	tucatinib 300 mg PO BID palbociclib 100 mg PO daily 21 day on, 7 days off letrozole 2.5 mg PO daily	Subjects 11-20 (if palbociclib toxicity increased)
DL -2	tucatinib 250 mg PO BID palbociclib 125 mg PO daily 21 day on, 7 days off letrozole 2.5 mg PO daily	Subjects 11-20 (if tucatinib toxicity increased)
DL -3	tucatinib 250 mg PO BID palbociclib 100 mg PO daily 21 day on, 7 days off letrozole 2.5 mg PO daily	Subjects 11-20 (if palbociclib and tucatinib toxicity increased, or unattributable toxicity)
DL -4	tucatinib 250 mg PO BID palbociclib 125 mg PO daily 21 day on, 7 days off letrozole 2.5 mg PO daily	Subjects 11-20 (if PKs indicate a significant change to the metabolism of tucatinib)*
DL -5	tucatinib 200 mg PO BID palbociclib 125 mg PO daily 21 day on, 7 days off letrozole 2.5 mg PO daily	Subjects 11-20 (if PKs indicate a significant change to the metabolism of tucatinib)*

*Significant PK changes regardless of noted clinical toxicities

To determine the safety of the triple combination, we will plan to enroll 10 subjects at the baseline starting doses (DL1) of tucatinib 300 mg PO BID, palbociclib 125 mg PO daily 21 days on and 7 days off, and letrozole 2.5 mg PO daily on a 28 day cycle length (Table 5).

After 10 subjects are accrued to the study and complete at least 1 cycle of treatment, we will perform an interim analysis of the safety phase Ib cohort. We anticipate that DLTs will likely be more attributable to palbociclib than tucatinib, but unbiased assessment of the toxicities will be performed. Based on the known rates of DLTs to date, as outlined above, the following rules will be applied:

10 subjects enrolled, completed at least one cycle of treatment (28 days) and evaluable for safety analysis:

If 7 or fewer subjects experienced DLTs of palbociclib, 3 or fewer subjects experienced DLTs of tucatinib, and 7 or fewer subjects experienced DLTs that are potentially attributable to both drugs, then the interim safety of the Phase Ib cohort will be considered acceptable and accrual will continue to enroll to 20 subjects without change of the starting doses. Otherwise, we will de-escalate to the next dose levels.

Scenario 1: Palbociclib toxicity increased

If 8 out of 10 or greater proportion of subjects experienced DLTs due to palbociclib, the palbociclib starting dose will be decreased to 100 mg PO daily 21 days on, 7 days off for the subsequent 10 subjects in the safety run in phase Ib cohort. Tucatinib starting dose will not be changed (DL -1).

Scenario 2: Tucatinib toxicity increased

If 4 out of 10 or greater proportion of subjects experienced DLTs due to tucatinib, we will proceed with a starting dose reduction of tucatinib to 250 mg PO BID and leave palbociclib starting dose unchanged (DL -2).

Scenario 3: Toxicity potentially attributable to both tucatinib and palbociclib:
If there is a lack of clarity or an apparent combination of DLTs that can be attributable to both tucatinib and palbociclib in 8 out of 10 or greater proportion of subjects, then a combined starting dose reduction of tucatinib to 250 mg PO daily and palbociclib to 100 mg PO daily 21 days on and 7 days off will occur (DL -3).

Incorporation of PKs in safety analysis:
Both tucatinib and palbociclib PK data will be considered during the first interim safety analysis. Based on the information available prior to phase I of the study, we did not expect significant changes in palbociclib PKs. With palbociclib being a weak time dependent CYP3A4 inhibitor, there was a possibility the tucatinib PKs will be changed. Therefore, the dose levels were designed accordingly: if PKs are suggesting a significant change to the metabolism of tucatinib, adjusting to the lower dose levels (DL -4 and DL -5) regardless of noted clinical toxicities was planned.

Based on the existing data for these drugs and the non-overlapping toxicity profiles, it is not anticipated that greater than one dose reduction of either tucatinib, or palbociclib, or both drugs will need to occur to determine the safe combination of drugs to move forward into phase II. If accrual to subjects 11-20 of the phase Ib cohort demonstrates a level of DLTs equal or above the threshold of 15/20 subjects for palbociclib attributable events, 7/20 subjects with tucatinib attributable events, or 14/20 subjects where DLTs are potentially attributable to both drugs (Table 4), or if PKs for subjects 11-20 are suggestive of clinically significant changes in the drug metabolism, then consideration of additional drug specific dose reductions will occur. It could potentially trigger enrollment of additional 10 subjects in the phase Ib safety cohort, or stopping clinical trial for the safety reasons, which will be determined, if this unlikely event should occur, by the by the Lead PI with the input from the study research team, the sponsor, Pfizer Inc., Seattle Genetics, Inc., and/or the protocol Data Safety Monitoring Committee.

Letrozole dose will be kept constant for all study subjects. Worsening of estrogen withdrawal symptoms are not anticipated to be increased through the drug combinations nor meaningfully effect the safety assessment of the protocol, as the timing of onset of many AI-based symptoms occurs months to years into the treatment. However, if a subject develops known AI dependent AEs of grade ≥3 meeting criteria for DLT, which we expect will occur at a frequency of 4-6% [20], the subject may choose to discontinue AI therapy. Subjects who discontinue letrozole may opt to remain on protocol if they continue taking both tucatinib and palbociclib, and if it is felt to be in their best clinical interest.

Attribution of DLTs to a specific study drug (tucatinib, palbociclib or letrozole) is described in detail in the section 6.2. A DLT experienced by a study subject will always necessitate dose modification and/or interruption of therapy with the study drug(s) for this subject. Dose modifications of the study drugs are described in section 6.3 of the protocol.

The study has completed the final phase Ib safety analysis on January 31,2019. Based on the data from the final phase Ib safety analysis, it has been determined that the study did not cross safety thresholds, and full doses of tucatinib, palbociclib and letrozole are reasonably well tolerated. Therefore, the recommended phase II doses (RP2D) were declared as tucatinib 300 mg

PO BID; palbociclib 125 mg PO daily 21 days on followed by 7 days off; and letrozole 2.5 mg PO daily (DL1).

However, after the study was re-opened for phase II on January 31,t 2019, new information on tucatinib became available via Safety Letter from Seattle Genetics sent to investigators on February 13 2019 ("Re: Safety Communication: Potential Risk of Drug-Drug Interaction"). This letter was to inform the investigators about potential drug-drug interaction between tucatinib and sensitive CYP3A4 substrates (such as palbociclib) based on the results of drug-drug interaction study ONT-380-012. This study demonstrated that in healthy volunteers tucatinib increased the geometric mean of midazolam exposure (AUC) 5.85-fold (90% CI 5.14 – 6.66); therefore, tucatinib belongs to the class of strong CYP3A4 inhibitors. According to the FDA prescribing information, the dose of palbociclib, which is a sensitive CYP3A4 substrate, needs to be decreased to 75mg PO daily 21 days on, 7 days off when palbociclib is used with strong CYP3A4 inhibitors. These findings were discussed with both Pfizer and Seattle Genetics, decision was made to change the dose of palbociclib to 75mg daily for all patients who were on study, and for all newly enrolled patients (as discussed in the Letter to the Investigators from the lead study PI Dr. Shagisultanova dated February 15 2019). Therefore, starting from February 15 2019, all new patients are enrolled in phase II part of the study at the following drug doses: tucatinib 300 mg PO BID; palbociclib 75 mg PO daily 21 days on followed by 7 days off; and letrozole 2.5 mg PO daily. For all patients who were on study prior to February 15 2019, the dose of palbociclib was changed to 75mg PO daily 21 days on followed by 7 days off (regardless of clinical toxicities), while the doses of tucatinib and letrozole were not changed. Additional PK analysis for palbociclib and tucatinib was planned to assure safety of the participants.

4.3 Efficacy analysis of the combination therapy

During the study, subjects will undergo standard radiologic imaging for complete assessment of disease to include chest, abdomen and pelvis, as well as bone assessment and appropriate imaging of any other known sites of disease. Recommended imaging includes a diagnostic quality CT scan and bone scan. Restaging brain MRI with contrast is required for subjects with brain metastases enrolled in the study. Imaging studies will be repeated every 8 weeks for the first 24 weeks, and then every 12 weeks thereafter, irrespective of dose holdings or interruptions. The primary efficacy endpoint for the phase II part of the study is PFS, or, for subjects with CNS disease enrolled into the study, bi-compartmental PFS (in the CNS and non-CNS compartments) defined as the time from allocation to the first documented disease progression according to RECIST 1.1 and/or RANO-BM criteria (Appendix C), or death due to any cause, whichever occurs first. If a subject does not have a documented date of progression or death, PFS will be censored at the date of the last adequate assessment. Subjects from both phase Ib and phase II part of the study will be included into a final efficacy analysis.

In this trial, if a subject is found to have progressive disease solely due to CNS progression per RANO-BM criteria and has a response to therapy outside of the CNS, the subject may stay on study provided this subject undergoes local therapy (radiotherapy or surgery) to the progressive CNS lesion(s). The subject may continue on study until a second progressive disease event occurs (either CNS or non-CNS). Such cases should be discussed with and receive approval from the Lead study PI at the time when initial CNS progression is documented.

4.4 Number of subjects

It is anticipated that 20 evaluable subjects in the phase Ib part and 20 subjects in the phase II part will be enrolled into this study. The phase II part of the study is designed to detect a 50% improvement in PFS comparing to historical control. TH3resa study (TDM-1 versus physician's choice therapy) enrolled similar patient population [33, 34] and represent appropriate study for comparison. TH3resa showed median PFS of 6.2 months in TDM-1 arm. Using the survival analysis and assuming that the survival time is exponentially distributed, accrual time is 12 months, follow-up time is 18 months, and drop out / loss of follow up rate is 5%, the sample size of 40 patients total (20 patients enrolled in phase Ib part, and 20 patients enrolled in phase II part) will allow us to achieve a statistical power of 82% with a one-sided type I error rate of 0.1 to detect 50% improvement in median PFS (from 6.2 month in the historical control [33, 34] to 9.3 months in the current study). Subjects who terminate participation for any reason before completing Cycle 1 are not considered evaluable and may be replaced within a timeframe of the study

4.5 Study timeline

- Phase Ib part accrual – 6 month
- Phase II part accrual – 6 month
- Total projected accrual time – 1 year
- Phase Ib primary end-point reporting – 8 month
- Phase II primary end-point reporting – 2.5 years
- Anticipated completion of the study – 2.5 years

This multicenter clinical trial will be conducted through the Academic Breast Cancer Consortium (ABRCC), with the University of Colorado Cancer Center as the lead site. A Protocol Contact Form will be maintained for the study and include a listing of all participating sites and key personnel.

5 ELIGIBILITY CRITERIA

This clinical trial can fulfill its objectives only if appropriate subjects are enrolled. The following eligibility criteria are designed to select subjects for whom protocol treatment is considered appropriate. All relevant medical and non-medical conditions should be taken into consideration when deciding whether this protocol is suitable for a particular subject.

5.1 Inclusion criteria

1. Subjects must have a histologically confirmed diagnosis of HR+/HER2+ locally advanced unresectable or metastatic breast cancer. Estrogen or progesterone receptor positivity is defined by IHC according to the most recent ASCO/CAP guidelines [29]. HER2 positivity is defined by standard of care fluorescence in situ hybridization (FISH) and/or 3+ staining by IHC according to the most recent ASCO/CAP guidelines [30].
2. Measurable and/or evaluable disease per RECIST 1.1 criteria and/or RANO-BM criteria (Appendix C). Bone only disease is allowed.
3. CNS inclusion criteria:
    - Subjects without CNS metastases are eligible. Note: brain imaging is not required for asymptomatic subjects without known brain metastatic disease prior to enrollment into the study

- Subjects with untreated asymptomatic CNS metastases not needing immediate local therapy in the opinion of investigator are eligible. For subjects with untreated asymptomatic CNS lesions > 2.0 cm by contrast-enhanced MRI, discussion with and approval from the Lead PI is required prior to enrollment
- Subjects with stable brain metastases previously treated with radiation therapy or surgery are allowed to enroll, provided that they are off corticosteroids or on stable/tapering dose of corticosteroids and stability of CNS metastatic disease for at least 4 weeks has been demonstrated, with the last MRI taken within 2 weeks prior to cycle 1 day 1 of the study. Relevant records of any CNS treatment must be available to allow for classification of target and non-target lesions

4. Age ≥ 18 years
5. ECOG performance status 0-1
6. Life expectancy of more than 6 months, in the opinion of the investigator
7. Study subjects should be post-menopausal women; premenopausal women are eligible if on ovarian suppression, or agreeable to mandatory ovarian suppression. Women of childbearing potential, defined as premenopausal women who are not permanently sterile (i.e., due to hysterectomy, bilateral oophorectomy, bilateral tubal ligation, or bilateral tubal occlusion) are required to have negative pregnancy tests prior to initiation of treatment.
8. Prior treatments:
   - Subjects should have received at least two approved HER2-targeted agents (trastuzumab, pertuzumab, or TDM-1) in the course of their disease
   - Subjects should have had at least 1 line of prior HER2-targeted therapy in the metastatic setting, with the exception of asymptomatic subjects with oligometastatic or bone / soft tissue only disease who, on investigator opinion, are appropriate for a single agent anti-endocrine therapy per NCCN guidelines
   - Subjects who have had up to 2 lines of prior endocrine therapy in the metastatic setting are allowed. Prior adjuvant and/or neoadjuvant endocrine regimens are allowed and not counted towards this limit
9. Adequate organ and marrow function as defined below:
   - Absolute neutrophil count ≥ 1,500/mm^3
   - Platelets ≥ 75,000/mm^3
   - Hemoglobin ≥ 9.0 mg/dL without red blood cell transfusion ≤ 7 days prior to Cycle 1 Day 1 of therapy
   - Total serum bilirubin ≤ 1.5 X upper limit of normal (ULN) except for subjects with known Gilbert's disease, who may enroll if the conjugated bilirubin is ≤ 1.5 ULN
   - AST (SGOT)/ALT (SGPT) ≤2.5 X ULN;
   - Serum creatinine ≤ 1.5 mg/dL
   - International normalized ratio (INR) and activated partial thromboplastin time (aPTT) ≤ 1.5 X ULN unless on medication known to alter INR and aPTT
   - Left ventricular ejection fraction (LVEF) ≥ 50% (as assessed by ECHO or MUGA) documented within 4 weeks prior to first dose of study treatment
   - Serum or urine pregnancy test (for women of childbearing potential) negative ≤ 7 days of starting treatment
10. Ability to understand and the willingness to sign a written informed consent and comply with the study scheduled visits, treatment plans, laboratory tests and other procedures.

11. Subject or legally authorized representative of a subject must provide signed informed consent per a consent document that has been approved by an institutional review board or independent ethics committee (IRB/IEC) prior to initiation of any study-related tests or procedures that are not part of standard-of-care for the subject's disease.

5.2 Exclusion criteria

1. Subjects with previously treated progressing brain metastases are excluded from the study
2. Subjects with known brain metastases and contraindications to undergo contrast MRI imaging of the brain are excluded from the study
3. Pregnancy or breast feeding
4. Current active treatment with an investigational agent
5. Known history of hypersensitivity to aromatase-inhibitor drugs
6. Any toxicity related to prior cancer therapies that has not resolved to ≤ Grade 1, with the exception of peripheral neuropathy, which must have resolved to ≤ Grade 2, and alopecia
7. Previous treatment with lapatinib, neratinib, afatinib, tucatinib, or other investigational EGFR-family receptor tyrosine kinase inhibitor or HER2 tyrosine kinase inhibitor.
8. Previous treatment with palbociclib, abemaciclib, ribociclib or other investigational CDK4/6 inhibitors
9. Any systemic anti-cancer therapy (including hormonal therapy), radiation, or experimental agent ≤ 2 weeks of first dose of study treatment
10. Active bacterial, fungal or viral infections requiring treatment with IV antibiotic, IV anti-fungal, or IV anti-viral drugs
11. Known active hepatitis B (HBV), hepatitis C (HCV) or human immunodeficiency virus (HIV) infections. Note: pretesting is not required.
12. Inability to swallow pills or any significant gastrointestinal disease which would preclude the adequate oral absorption of medications
13. Use of prohibited medications listed in Appendix D within 3 elimination half-lives of the inducer or inhibitor prior to first dose of the study treatment
14. Known myocardial infarction, severe/unstable angina, percutaneous transluminal coronary angioplasty/stenting (PTCA), or coronary artery bypass graft (CABG) within 6 month of the first dose of the study treatment
15. Clinically significant cardio-vascular disease, such as ventricular arrhythmia requiring therapy, uncontrolled hypertension (defined as persistent systolic blood pressure > 160 mm Hg and/or diastolic blood pressure > 100 mm Hg on antihypertensive medications), or any history of symptomatic CHF
16. Other severe acute or chronic medical or psychiatric conditions or laboratory abnormalities that may increase the risk associated with study participation or study drug administration, or may interfere with the interpretation of study results, or in the judgment of the investigator would make the subject inappropriate for entry into the study.

6 STUDY TREATMENTS

6.1 Drug Supply

6.1.1 Tucatinib formulation, packaging and storage

Tucatinib is manufactured as tablets 150mg and 50mg. It is available as both a coated yellow oval-shaped tablet in a 150 mg dosage strength and a coated yellow round convex tablet in a 50 mg dosage strength for PO administration. The tablets are manufactured from a drug product

intermediate amorphous dispersion of tucatinib in polyvinylpyrrolidine-vinyl acetate copolymer (PVP-VA), which is then combined with standard pharmaceutical excipients and compressed into tablets. The tablets are packaged in round, high-density polyethylene bottles containing a desiccant, with an induction sealed liner and child-resistant plastic closure cap.

Tucatinib is stored under refrigeration (2 to 8°C) and should be handled with care. Stability studies to support the drug storage conditions have been conducted. The shelf life / expiration date of each lot of the drug and appropriate storage conditions will be closely monitored.

Tucatinib will be supplied by Seattle Genetics, Inc.

6.1.2 Palbociclib formulation, packaging and storage

Palbociclib is manufactured in tablets of 125 mg, 100 mg, and 75 mg. Palbociclib is supplied in the following strength and package configurations (Table 6):

Table 6. Palbociclib formulations

Package Configuration	Tablet Strength (mg)	NDC	Tablet Description
Blister packs of 21 tablets	125	NDC 0069-0189-21	Smooth-coated tablet sored in a white and green box stamped with the word "IBRANCE".
Blister packs of 21 tablets	100	NDC 0069-0188-21	Smooth-coated tablet, stored in a white and purple box stamped with the word "IBRANCE".
Blister packs of 21 tablets	75	NDC 0069-0187-21	Smooth-coated tablet, stored in a white and blue box stamped with the word "IBRANCE".

Palbociclib should be store at 20ºC to 25ºC (68ºF to 77ºF); excursions permitted between 15ºC to 30ºC (59ºF to 86ºF).

Palbociclib will be supplied by Pfizer Inc.

6.1.3 Letrozole formulation, packaging and storage

Letrozole is manufactured as 2.5 mg tablets. Letrozole tablets are dark yellow, film-coated, round, slightly biconvex, with beveled edges, imprinted with the letters FV on one side and CG on the other side. Letrozole is packaged in HDPE bottles with a safety screw cap containing 30 tablets (NDC 0078-0249-15). Letrozole should be store at 25°C (77°F); excursions permitted to 15-30°C (59-86°F).

Letrozole will be obtained commercially per standard of care.

6.1.4 Drug accountability

Upon receipt at the investigative site, palbociclib and tucatinib should be stored according to the manufacturer instructions in the original packaging. Tucatinib should be stored under refrigeration at 2 to 8°C. Palbociclib showed be stored at 20 and 25°C (excursions permitted between 15 and 30°C). The drugs should be protected from excessive humidity in a monitored, locked, secure area with limited access. Storage area temperature conditions must be monitored and recorded daily. All temperature excursions will be reported to the sponsor for assessment and authorization for continued use. Study site staff must instruct subjects on how to store and administer oral medications that will be dispensed for at-home administration.

Accountability for study drug product is the responsibility of the investigator. The study site must maintain accurate records demonstrating dates and amount of study treatment received, to whom it was dispensed (subject-by-subject accounting), and accounts for any palbociclib and tucatinib accidentally or deliberately destroyed. A written explanation must be provided for any discrepancies. Subjects are to be instructed on proper accountability of the take-home study drugs and will be instructed to return any unused drug in the original packaging at the appropriate clinic visits. The investigator must return all unused drug products provided by Pfizer Inc. and Seattle Genetics, Inc.

6.1.5 Administration

Tucatinib should be taken orally twice a day approximately at the same time. Palbociclib should be taken orally once daily for 21 consecutive days at approximately same time of the day, followed by 7 days off treatment to comprise a complete cycle of 28 days. Palbociclib can be taken with proton pump inhibitors (PPI)/antacids and does not contain lactose or gelatin. Palbociclib tablets should be swallowed whole (participants should not chew, crush or open them prior to swallowing). Letrozole should be taken once daily in the dose of 2.5 mg approximately at the same time of the day.

All three medications may be taken with or without food (without regards to meals), because food does not influence absorption of the tablets of tucatinib, palbociclib or letrozole. However, to prevent stomach discomfort that sometimes may be caused by taking medications in the tablet form, for this study the Investigator recommends taking all study medications with food.

Subjects will be instructed by the study team designate of the Investigator as to the specific dose of tucatinib and palbociclib in accordance with the appropriate DL. Complete dosing instructions will be provided to study subjects, and will include the minimum times between doses, dosing relation to meals, and instructions for missed doses. Subjects will receive study treatments on 28 day cycles until unacceptable toxicity, disease progression, withdrawal of consent, or study closure.

6.1.6 Compliance

Compliance for this study is defined as taking ≥ 80% of the intended dose in any given cycle (28 day period). Any dose delay within the 28 day-period represents compliance days missed. The achieved dose intensity is irrespective of sequential or intermittent missed dosing. Compliance will be assessed on a subject-by-subject basis. The pharmacist or designee will record the number of palbociclib tablets and tucatinib tablets dispensed to each individual subject, and the number of palbociclib tablets and tucatinib tablets returned to the clinic at the end of each cycle. Data regarding the administration of letrozole will also be collected by the site after each cycle. Dose modifications and interruptions of any study drug will be documented in the source documents and the case report forms (CRFs).

6.2 Attribution of toxicities

Palbociclib, tucatinib and letrozole have largely non-overlapping toxicity profiles. Therefore, dose modifications or discontinuation of the study drugs will be performed taking into account specific toxicities of these agents. If a subject on the study develops specific toxicity attributable to one of the study drugs, only the dose of this particular drug will be modified (as described further in the section 6.3). Subjects are allowed to stay on the study if either palbociclib or letrozole are discontinued for toxicity. If both palbociclib and letrozole are stopped for toxicity, a subject will be removed from the study. If tucatinib is discontinued for toxicity, a subject will be

removed from the study. Tables 7, 8 and 9 summarize common, less common and rare side effects of tucatinib, palbociclib and letrozole (presented in the alphabetical order).

Table 7. Toxicity profile of tucatinib

Common side effects (>20%)	Less common side effects (1-20%)	Rare side effects (<1%)
Diarrhea Dizziness Fatigue Nausea Rash Weakness	Abdominal pain Alkaline phosphatase elevation ALT and AST elevation* Anemia Back pain Bilirubin elevation Constipation Cough Creatinine increase Decreased appetite Electrolyte abnormalities Headache Hypokalemia Hypomagnesemia Hyponatremia HypophosphatemiaMusculoskeletal chest pain Myalgia Night sweats Pain in limbs Peripheral edema Shortness of breath Vomiting Upper respiratory infections Urinary tract infections	Decreased LVEF Dehydration Symptomatic CHF** EKG changes/QT prolongation Arrhythmias

* Cases of grade 4 (potentially life threatening) ALT and AST elevation with tucatinib treatment has been reported; ** Symptomatic CHF manifests as clinical symptoms of fluid retention, tachycardia, and shortness of breath in the settings of elevated BNP and / or ECHO findings diagnostic of CHF

Table 8. Toxicity profile of palbociclib

More Common toxicities (>30%)	Common toxicities (10-30%)	Less common toxicities (5-10%)	Rare toxicities (<5%)
Fatigue Infections* Leukopenia Lymphopenia Neutropenia	Anemia Arthralgia Back pain Common Cold Constipation Cough Diarrhea	Abdominal pain Abnormal liver tests Anxiety Asthenia (weakness) Chest pain Depression Dry mouth	Blurred vision Dry eyes or watery eyes Interstitial lung disease (pneumonitis) Febrile neutropenia

	Dizziness Decreased appetite Dyspnea (shortness of breath) Insomnia Fever Headache Hair loss Hot flash Mucositis / stomatitis Nausea Pain in the limbs Thrombocytopenia Vomiting Rash	Dry skin Dysgeusia (taste changes) Epistaxis Increased creatinine Indigestion Falls Flu-like symptoms Heartburn Hypertension Mouth ulcers Myalgia Muscle cramps Pain Peripheral edema Pruritus	

*Infections include serious bacterial or viral infections, or reactivation of latent infections. A case of mycobacterial infection (tuberculosis) was reported on treatment with palbociclib.

Table 9. Toxicity profile of letrozole

Common toxicities (>20%)	Less common toxicities (1-20%)	Rare toxicities (<1%)
Arthralgias Hot flashes Mood swings Myalgias Vaginal dryness	Dizziness Fatigue Headache Hypertension Insomnia Increased blood glucose Increased cholesterol Nausea Night sweats Osteoporotic bone fractures Urinary tract infections Vaginal bleeding Vomiting Weight gain Worsening bone mineral density	Constipation

Attribution of toxicities will be done per investigator opinion, and in consultation with the Lead PI in case of SAEs. Table 10 can be used as a guidance for attribution of toxicities, however, investigators are encouraged to use their best clinical judgement.

Table 10. Suggested attribution of toxicities to study drugs

Toxicities very likely attributable to		
tucatinib	**palbociclib**	**letrozole**
ALT and AST elevation[a] Alk Phos elevation Elevation of bilirubin Decreased LVEF Symptomatic CHF	Anemia Bacterial or fungal infections (in the settings of grade ≥3 neutropenia) Dry eyes or watery eyes Dysgeusia Epistaxis Hair loss Leukopenia Lymphopenia Mucositis / stomatitis Neutropenia Neutropenic fever Thrombocytopenia	Arthralgias Hot flashes Mood swings Myalgias Night sweats Osteoporotic bone fractures UTIs (in the settings of vaginal dryness) Vaginal dryness Worsening bone density
Toxicities potentially attributable to both tucatinib and palbociclib		
Anemia Anorexia Constipation Diarrhea Dizziness Electrolyte abnormalities Fatigue Headaches	Myalgias Nausea Pain in the limbs Peripheral edema Pruritus Rash Vomiting Weakness Upper respiratory tract infections	

[a] Although LFT elevation is much more likely to be attributed to tucatinib, palbociclib may contribute. Therefore, **in all cases of bilirubin or ALT / AST elevation of ≥G3, both tucatinib and palbociclib should be put on hold, and lead PI should be consulted regarding the attribution of toxicity.**

In cases of grade ≥3 toxicities potentially attributed to both tucatinib and palbociclib, doses of both tucatinib and palbociclib will be decreased (as described in the section 6.3.3). Alopecia, grade 3 fatigue lasting ≤7 days, grade ≥3 electrolyte abnormalities returning to grade ≤1 within 72 hours spontaneously or with optimal medical management, grade 3 rash in the absence of adequate management with anti-infectives and / or topical corticosteroids that improves with optimal medical management in <72hrs, grade 3 nausea, vomiting, or diarrhea in the absence of optimal use of anti-emetic or antidiarrheal medications that improves with optimal medical management in < 72hrs, and grade ≥3 amylase or lipase elevation that is not associated with symptoms or clinical manifestations of pancreatitis do not require dose modifications of the study drugs.

6.3 Dose modifications of the study drugs

6.3.1 Dose modification of tucatinib

Tucatinib will be administered at 300 mg PO BID, which is the current MTD/RP2D when given in combination with trastuzumab, ado-trastuzumab emtansine, or capecitabine. Any grade 3 toxicity clearly related to tucatinib (such as elevation of ALT, AST, and/or bilirubin) will result in the drug being held until the toxicity returns to grade ≤1. Tucatinib-related toxicity must resolve to grade ≤1 within 3 weeks for the subject to remain on study. Then, subject may resume therapy at 250 mg PO BID. A second and third dose hold for toxicity may occur with the timeline of 3 weeks for symptom recovery to grade ≤1, then the drug may be resumed at 200 mg and 150 mg BID, respectively. If a fourth episode of grade 3 tucatinib related toxicity occurs, drug should be held and subject removed from study. Once the dose of tucatinib is de-escalated for an individual subject, it will not be increased for that subject during later cycles. At any time if grade 3 toxicity is not resolved to grade ≤1 within 3 weeks, subject will be removed from study.

Any significant asymptomatic decline in LVEF requires additional assessment, holding of tucatinib, and management as outlined in Table 13. All subjects with an asymptomatic decline in EF to below 50% should be considered for medical management of LVEF decline associated with HER2-targeted therapy per standard guidelines. All incidence of symptomatic CHF will result in immediate discontinuation of tucatinib and the subject will be removed from study. All subject with symptomatic CHF should be followed until resolution of the event, even once removed from study.

Table 11. Dose modifications of tucatinib

Dose Modification for AEs	Dose
Recommended starting dose	300mg PO BID
First dose reduction	250 mg PO BID
Second dose reduction	200 mg PO BID
Third dose reduction	150mg PO BID

Dose reductions of greater increments than those listed in this table (i.e. more than 50 mg per dose reduction) may be made if considered clinically appropriate by the investigator. However, tucatinib dose may not be reduced below 150 mg BID. Additionally, for the starting dose levels lower than 300mg PO BID, the same dose modification tiers above will be followed, with the lowest dose allotted being 150mg PO BID.

Table 12. Dose modifications of tucatinib for liver function abnormalities [see also section 8.1.4 for details on events of special interest – drug-induced liver injury]

Liver function abnormalities	Action for tucatinib
Grade 2 elevation of ALT and/or AST (> 3 – ≤ 5 x ULN)	Dose modification not required
Grade 3 elevation of ALT and/or AST (> 5–20 x ULN)[a]	Hold until severity ≤ Grade 1. Restart at the next lower dose level
Grade 4 elevation of ALT and/or AST (> 20 x ULN)	Discontinue drug

Elevation of ALT and/or AST (> 3 x ULN) AND Bilirubin (> 2 x ULN) **[See section 8.1.4 on events of special interest – drug-induced liver injury]**	Discontinue drug
Grade 2 elevation of bilirubin (> 1.5–3 x ULN) AND both ALT and AST (< 3 x ULN)	Hold until severity ≤ Grade 1. Restart at same dose level
Grade 3 elevation of bilirubin (> 3 – ≤ 10 x ULN) AND both ALT and AST (<3 x ULN) [a]	Hold until severity ≤ Grade 1. Restart at the next lower dose level
Grade 4 elevation of bilirubin (> 10 x ULN)	Discontinue drug

[a] Although LFT elevation is much more likely to be attributed to tucatinib, palbociclib may contribute. Therefore, **in all cases of bilirubin or ALT / AST elevation of≥G3, both tucatinib and palbociclib should be put on hold, and lead PI should be consulted regarding the attribution of toxicity.** Abbreviations: alanine aminotransferase (ALT); aspartate aminotransferase (AST); upper limit of normal (ULN).

Table 13. Dose modifications of tucatinib for left ventricular dysfunction

LVEF / CHF	Tucatinib dose modification
LVEF ≥ 50%	Continue treatment with tucatinib.
LVEF 40% to <50% and decrease is ≤ 15% absolute decrease from pretreatment baseline	Continue treatment with tucatinib. Repeat LVEF assessment within 3 weeks. If LVEF has worsened, or not recovered to ≥ 50% or within 15% of absolute change from baseline, hold tucatinib and repeat LVEF assessment within 3 weeks. Consider medical management as per HER2 target therapy standard guidelines.
LVEF 40% to <50% and ≥16% absolute decrease from pretreatment baseline	Do not administer tucatinib. Repeat LVEF assessment within 3 weeks. Consider standard medical management of HER2 therapy-induced LVEF decline. If the LVEF has not recovered to ≤15% absolute change from baseline, discontinue tucatinib and remove subject from the study.
LVEF < 40%	Do not administer tucatinib. Repeat LVEF assessment within 3 weeks and consider standard medical management of HER2 therapy-induced LVEF decline. If LVEF < 40% is confirmed, discontinue tucatinib and remove from study. If LVEF is improved to ≥40%, continue to follow the algorithm in this table.
Symptomatic CHF*	Discontinue tucatinib

Abbreviations: Congestive Heart Failure (CHF); Left Ventricular Ejection Fraction (LVEF).
*Symptomatic CHF manifests as clinical symptoms of fluid retention, tachycardia, and shortness of breath in the settings of elevated BNP and / or ECHO findings diagnostic of CHF

6.3.2 Dose modifications of palbociclib

Since new information became available about potential drug-drug interaction between tucatinib and palbociclib, different dose modifications were used prior to February 15, 2019, and after February 15, 2019.

Dose modifications of palbociclib prior to February 15 2019 (prior to availability of safety information on potential tucatinib – palbociclib interaction)

Palbociclib will be initiated at the FDA approved dose of 125 mg PO daily 21 days on, 7 days off. Dose interruption and modifications for known palbociclib-related AEs (including bone marrow suppression, neutropenic fever, bacterial or fungal infections in the settings grade ≥3 neutropenia, mucositis / stomatitis) should occur per palbociclib full prescribing information brochure [28]. Palbociclib should be held for any subject who experiences a grade ≥ 3 non-hematologic toxicities clearly related to palbociclib, grade 3 neutropenia with fever, or grade 4 neutropenia, and resumed when symptoms improved to grade ≤2 at the next dose level. Palbociclib should be discontinued if no improvement of symptoms is seen within 3 weeks. Up to two dose reductions of palbociclib are allowed (100 mg, and 75 mg PO daily). Palbociclib dose should not be re-escalated for a subject after a dose reduction is made. Subject should discontinue palbociclib if a delay greater than three weeks is required due to treatment-related toxicity of palbociclib. Subjects may remain on study after discontinuation of palbociclib, if both letrozole and tucatinib are continued.

Table 14. Dose modifications of palbociclib

Dose level	Dose
Recommended starting dose	125 mg/day
First dose reduction	100 mg/day
Second dose reduction	75 mg/day

For the starting dose levels lower than 125mg/day, the same dose modification tiers above will be followed, with the lowest dose allotted being 75 mg/day.

Dosing of palbociclib after February 15, 2019 (after the information on potential tucatinib – palbociclib interaction became available)

Since the information became available on potential drug-drug interaction between tucatinib and palbociclib, palbociclib will be dosed at 75mg PO daily 21 days on, 7 days off for all study participants. No dose reduction levels will be allowed. Palbociclib will be held or discontinued for significant or unresolving toxicities as specified in tables 15 and 16. Patients who discontinued palbociclib may remain on study, as long as they continue to receive tucatinib and letrozole.

Table 15. Palbociclib dose modifications for hematologic toxicities

Hematologic toxicities[a]	Dose modifications

Grade 1 or 2	No dose adjustment is required.
Grade 3[b]	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/mm3) + Fever ≥38.5ºC and/or infection	Withhold palbociclib and initiation of next cycle until recovery to Grade ≤2 (≥1000/mm3). Resume at the next lower dose [c].
Grade 3 thrombocytopenia with bleeding	Withhold palbociclib and initiation of next cycle until recovery to Grade ≤2 and cessation of bleeding. Resume at the next lower dose [c].
Grade 4[b]	Withhold palbociclib and initiation of next cycle until recovery to Grade ≤2. Resume at the next lower dose [c].

Abbreviations: Absolute Neutrophil Count (ANC)
a - Monitor complete blood count prior to the start of palbociclib therapy and at the beginning of each cycle, as well as on Day 15 of the first two cycles, and as clinically indicated. *b* – Except lymphopenia (unless associated with clinical events, e.g., opportunistic infections). *c* – If patient is on the lowest possible dose of 75mg PO daily, palbociclib should be discontinued.

Table 16. Palbociclib dose modifications for non-hematologic toxicities

Non-Hematologic Toxicities	Dose Modifications
Grade 1 or 2	No dose adjustment is required.
Grade ≥3	Withhold until symptoms resolve to: • Grade ≤1; • Grade ≤2 (if not considered a safety risk for the subject) Resume at the next lower dose [a].

a – If patient is on the lowest possible dose of 75mg PO daily, palbociclib should be discontinued.

6.3.3 Dose modifications of tucatinib and palbociclib for toxicities potentially attributable to both drugs

Examples of toxicities that might be attributed to both tucatinib and palbociclib include constipation, diarrhea, dizziness, fatigue, nausea and rash. Both palbociclib and tucatinib should be held for any subject who experiences a Grade ≥ 3 AE related to the combination of tucatinib with palbociclib (as determined by the treating clinician and/or Investigator), and both drugs should be restarted at the next dose level if toxicity resolve to grade ≤1 within 3 weeks. Alopecia, grade 3 fatigue lasting ≤7 days, grade ≥3 electrolyte abnormalities returning to grade ≤1 within 72 hours spontaneously or with optimal medical management, grade 3 rash in the absence of adequate management with anti-infectives and / or topical corticosteroids that improves with medical management in <72hrs, grade 3 nausea, vomiting, or diarrhea in the absence of optimal use of anti-emetic or antidiarrheal medications that improves with optimal medical management in < 72hrs, and grade ≥3 amylase or lipase elevation that is not associated with symptoms or clinical manifestations of pancreatitis do not require dose modifications of the study drugs.

Table 17. Dose Modifications of tucatinib and palbociclib for toxicities that may be attributable to both drugs

Toxicities potentially related to both tucatinib and palbociclib	**Dose modifications of tucatinib and palbociclib**
≥ Grade 3 AEs (other than grade 3 fatigue lasting ≤ 7 days; alopecia; nausea; vomiting; diarrhea; rash; correctable electrolyte abnormalities returning to ≤grade 1 in ≤ 72hrs).	Hold until severity ≤ grade 1 or pre-treatment level, restart at the next lower dose level [a]
Grade 3 nausea, vomiting, or diarrhea WITHOUT optimal use of anti-emetics or anti-diarrheals	Hold until severity ≤ grade 1 or pre-treatment level. Initiate appropriate therapy. Restart without dose reduction if symptoms improve with optimal medical management in < 72hrs (otherwise, restart at the next lower dose level [a]).
Grade 3 nausea, vomiting, or diarrhea WITH optimal use of anti-emetics or anti-diarrheals.	Hold until severity ≤ grade 1 or pre-treatment level. Restart at the next lower dose level [a].
Grade 4 nausea, vomiting, or diarrhea regardless of use of anti-emetics or anti-diarrheals.	Do not administer until severity ≤ grade 1. Restart at the next lower dose level [a].
Grade 3 rash WITHOUT optimal use of topical corticosteroids or anti-infectives.	Hold until severity ≤ grade 1 or pre-treatment level. Initiate appropriate therapy. Restart without dose reduction if symptoms improve with optimal medical management in < 72hrs (otherwise, restart at the next lower dose level [a]).
Grade 3 rash WITH optimal use of topical corticosteroids or anti-infectives.	Hold until severity ≤ grade 1 or pre-treatment level. Restart at the next lower dose level [a].
Grade 4 rash regardless of use of topical corticosteroids or anti-infectives.	Permanently discontinue study drugs.
Alopecia (any grade)	No dose modifications
Grade 3 fatigue lasting ≤7 days	No dose modifications
Grade ≥3 electrolyte abnormalities returning to ≤ grade 1 within 72hrs spontaneously or with optimal medical management	No dose modifications (if no improvement within 72hrs, hold until severity improves to grade ≤1, restart at the next lower dose level)

a – If patient is on the lowest possible dose level, the drug should be discontinued.

6.3.4 Discontinuation of letrozole

Letrozole will be administered at fixed dose 2.5mg PO daily with no dose adjustments. Premenopausal women enrolled in the study will be required to receive ovarian function suppression (goserelin 3.8 mg subcutaneously every 28 days is the recommended method; however, any standard drug and drug frequency for ovarian function suppression is allowed per discretion of treating physician with Lead PI approval). Letrozole-related AEs include hot flashes, arthralgias, myalgias, mood swings, vaginal dryness and urinary tract infections in the settings of vaginal dryness. Letrozole may be held for grade 3 toxicity clearly or potentially attributable to this drug, as determined by the treating physician. When symptoms resolve to ≤ grade 2, the subject

may resume letrozole. If symptoms do not resolve to grade ≤2 within 3 weeks, or if the second episode of grade 3 toxicity occurs, letrozole should be discontinued. Subjects will be allowed to discontinue letrozole and remain on study if they continue taking both tucatinib and palbociclib and derive benefits from this therapy.

6.4 Concomitant medications

All concomitant medications will be recorded in the electronic CRFs. Concomitant medications can be administered at the investigator's discretion to conform to standard practice during the treatment period.

6.4.1 Potential drug interactions

Tucatinib is metabolized by CYP450 enzymes in human liver, primarily by CYP2C8 and to a lesser extent CYP3A4. To minimize the risk of potential drug-drug interaction, concurrent use of strong CYP2C8 and CYP3A4 inhibitors or inducers (listed in the Appendix D), will not be allowed during the study period. Moderate CYP2C8 inhibitor trimethoprim sulfate is also prohibited in combination with tucatinib due to a case of increased LFTs in prior clinical trials. Moderate inhibitors or inducers of CYP3A4, as well as moderate inhibitors of CYP2C8 other than trimethoprim (listed in the Appendix D) should be used with caution.

New information on tucatinib inhibition of CYP3A4 became available from the study ONT-380-012. Preliminary PK results from this study indicated that coadministration of multiple doses of tucatinib (300 mg BID) with midazolam (a sensitive CYP3A substrate) increased the geometric mean midazolam exposure (AUC) approximately 5.85-fold (90% CI 5.14 to 6.66) in healthy subjects, compared with administration of midazolam alone. **Therefore, tucatinib is a strong CYP3A4 inhibitor, and its use together with sensitive CYP3A4 substrate should be avoided, or doses of sensitive CYP3A4 substrates should be adjusted accordingly.**

Our safety analysis of phase Ib part of the study did not indicated excessive palbociclib toxicity or significant changes of PKs up to 8 hrs post administration of palbociclib with tucatinib. However, in the interest of safety of all participants, dosing of palbociclib for all patients enrolled in the study was changed to 75mg PO daily 21 days on, 7 days off (FDA approved dosing of palbociclib when co-administered with strong CYP3A4 inhibitors), once information of potential drug-drug interaction became available. Additional PKs will be obtained at late time points from 10 patients enrolled in phase II part of the study as discussed in chapter 8.3 and table 19.

Palbociclib is primarily metabolized by CYP3A and sulfotransferase (SULT) enzyme SULT2A1. Because of these, strong CYP3A inhibitors and inducers (listed in the Appendix D) will not be allowed during the study. Palbociclib is a weak time-dependent inhibitor of CYP3A following daily 125 mg dosing to a steady state. Administration of sensitive CYP3A4 substrate midazolam with multiple doses of palbociclib increased midazolam plasma exposure by 61% in healthy subjects, compared with administration of midazolam alone [28], which is consistent with palbociclib being a weak CYP3A4 inhibitor according to FDA drug interaction labeling (http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#table3-1). Therefore, sensitive CYP3A4 substrates with low therapeutic index (listed in the Appendix D) should be used with caution during study period.

Palbociclib is FDA approved to be co-administered with letrozole, which is a weak CYP3A4 substrate. Co-administration of palbociclib with letrozole did not result in clinically significant drug interaction.

Letrozole is a minor substrate of CYP3A4 and CYP2A6 and a strong inhibitor of CYP2A6. Letrozole may increase the serum concentration of methadone, the dose of methadone may need to be adjusted. Clinically, letrozole does not display any significant interaction with palbociclib or HER2-targeted agents.

Table 18. Liver metabolism of tucatinib, palbociclib and letrozole

Drug	Substrate of	Inhibitor of
tucatinib	CYP2C8 CYP3A4 (minor)	CYP2C8, CYP2C9 (moderate) CYP2C19, CYP1A2, CYP2D6 (weak) CY3A4 (strong) [a]
palbociclib	CYP3A4 SULT	CYP3A (weak)
letrozole	CYP3A4 (minor) CYP2A6 (minor)	CYP2A6 (strong) CYP2C19 (weak)

a – Preliminary data from ONT-380-012 study

6.4.2 Allowed concomitant therapy

Subjects may continue to use any ongoing medications not prohibited by the inclusion and exclusion criteria. Efforts should be made to maintain stable doses of concomitant medications during the course of study treatment. During study treatment, subjects may receive supportive care to include:

- Bisphosphonates, denosumab
- Transfusions of blood and blood products
- Antibiotics (except trimethoprim/sulfamethoxazole and other prohibited medications listed in Appendix D)
- Pain medications
- Anti-diarrheals, anti-emetics, antacids, and laxatives
- Acetaminophen and NSAIDs may be used to manage fever, myalgias or arthralgias
- Anti-histamines may be used to manage drug-related AEs such pruritus
- Topical creams and emollients
- Vaginal estrogen preparations for relieve of vaginal dryness
- Thoracentesis or paracentesis may be performed, if needed for comfort
- Systemic anticoagulation with warfarin, heparin and low molecular weight heparins if required for prophylaxis or management of vascular thromboembolic events
- If surgical intervention or localized radiation become indicated (for palliation), these interventions are permitted for non-target lesions in situations where other disease remains evaluable by RECIST 1.1 and / or RANO-BM criteria (Appendix C).These interventions should be avoided if clinically feasible until after the second response assessment, and the Lead PI should be consulted prior to the intervention occurring. Investigational drugs must be held during surgery / radiotherapy and for 1 week afterwards. If possible, the investigationa1 drug should be held for 1 week prior to surgery and palliative radiotherapy.
- Chronic use of systemic corticosteroids should be limited to ≤ physiologic replacement doses (i.e. prednisone ≤10 mg/day or equivalent). Short courses of higher dose systemic steroids (to treat COPD, gout, rheumatic disorders, etc.), are permitted. Intra-articular

steroid injections are permitted. Use of non-systemic steroid use is permitted (e.g. cream, lotion, inhalers).

- The use of growth factors support (G-CSF, PEGylated G-CSF) must follow institutional and ASCO guidelines. Antibacterial and antifungal prophylaxis should be consistent with ASCO and institutional guidelines.

6.4.3 Prohibited concomitant therapy

The following therapies are prohibited during the study (unless otherwise noted):

- Investigational drugs and devices
- Anti-cancer therapy, including chemotherapy, targeted therapy other than tucatinib and palbociclib, and anti-hormonal therapy other than letrozole and agents for ovarian function suppression in premenopausal females
- Radiation therapy, except for palliative radiotherapy at focal sites (not considered target lesions per RECIST 1.1 and / or RANO-BM criteria) that may be given after consultation with the Lead PI, if there remain other sites for assessment of disease response
- The use of thrombopoietin agonists
- The use of erythropoietin
- Strong inhibitors or inducers of CYP2C8, and a moderate CYP2C8 inhibitor trimethoprim/sulfamethoxazole (see Appendix D)
- Strong inhibitors or inducers of CYP3A4 (see Appendix D)
  Note that sensitive substrates of CYP3A4 should be use with caution and close monitoring for side effects (see Appendix D)

7 STUDY PROCEDURES

The schedule of events is summarized in the Study Calendar Table. Study activities are listed by visit in this section. Descriptions of study assessments are presented in Section 8. All assessments performed on Day 1 of all treatment cycles should be performed prior to administration of study drugs, unless otherwise indicated. If the timing of a protocol-mandated procedure coincides with a holiday and/or weekend that precludes the procedure within the allotted window, the procedure should be performed on the nearest following date. Sites are required to submit an enrollment packet that will include a Subject Eligibility Checklist prior to enrollment of the subject. A confirmation of enrollment will be provided to the site by Criterium or designee.

7.1 Baseline

The following procedures/assessments must be performed within 28 days prior to the first dose of study drugs:

- Subject signature on informed consent form
- Documentation of study eligibility per inclusion/exclusion criteria
- Documentation of ER and /or PR positivity (defined by IHC according to the most recent ASCO/CAP guidelines) in the metastatic tumor biopsy (if metastatic tumor is not accessible for biopsy, documentation of ER and / or PR positivity in the primary tumor biopsy is acceptable)
- Documentation of HER2 positivity defined by fluorescence in situ hybridization (FISH) and/or 3+ staining by IHC according to the most recent ASCO/CAP guidelines in the metastatic tumor biopsy (if metastatic tumor was not biopsies, documentation of HER2 positivity in the primary tumor biopsy is acceptable)

- Baseline signs and symptoms
- Documentation of disease history, including year of initial breast cancer diagnosis, tumor grade and stage at initial diagnosis (if available), year of diagnosis of metastatic disease, location of known metastatic lesions, and all prior therapies
- Documentation of baseline medical and surgical conditions
- Documentation of concomitant medications
- Vital signs (blood pressure, heart rate and body temperature)
- Height (only needed for the initial baseline assessment)
- Weight
- Physical exam
- ECOG performance status
- Hematology, blood chemistry, liver function tests, coagulation tests (PT/INR and PTT)
- For females of childbearing potential – serum pregnancy test and plan for contraception on protocol. Women receiving ovarian suppression are recommended to receive counselling on additional forms of contraception to be used while on study treatment.
- Transthoracic ECHO or MUGA to assess LVEF (Note: that whichever testing modality is chosen in screening should be used for all subsequent cardiac assessments throughout the study for comparison)
- Diagnostic quality CT scan and bone scan to document all sites of disease and assess tumor burden based on RECIST 1.1 criteria. Brain MRI is not required for neurologically asymptomatic subjects without known brain metastases. Baseline brain MRI is required in subjects with neurological symptoms and/or known brain metastases to allow assessment of disease by RANO-BM.
- EKG
- Research blood sample for assessment of potential biomarkers of response (please refer to the Study Lab Manual for details)
- Pre-treatment tumor biopsy (optional, but highly encouraged)
- Collection of primary and / or metastatic tumor blocks and / or slides will be initiated after enrollment (please refer to the Study Lab Manual for details)

Following successful completion of the baseline assessments and confirmation of eligibility subjects may be enrolled.

7.2 Trial Period

7.2.1 Cycle 1 Day 1

- Documentation of AEs
- Documentation of concomitant medications
- Physical examination*
- Vital signs (heart rate, BP, temperature), body weight
- ECOG PS*
- Blood samples for hematology, clinical chemistry and liver function tests (results must be reviewed and eligibility confirmed prior to first dose of study drugs)*
- Dispense tucatinib and palbociclib (except patients enrolled in phase II part that have PK evaluation on cycle 1 day 9; in those patients tucatinib will be held and should not be dispensed until PK assessment is completed)

- For patients having PK analysis on cycle 1 day 9, recommend to take palbociclib and letrozole at PM time (otherwise – palbociclib and letrozole can be taken at any time during the day, as long as 24hr intervals between doses are maintained)

* These assessments do not need to be repeated if performed within 72 hours of C1D1.

7.2.2 Cycle 1 Day 8

- Patients enrolled in phase II part of the study who have PKs scheduled on the next day (day 9) should take high calorie / high protein meal at 8:00PM and palbociclib at 10:00PM. Phone call by coordinator is recommended to remind the patient about timing. Patient can take letrozole at any time during the day. Patient records timing of study drugs in the diary.

7.2.3 Cycle 1 Day 9

- Patients who are part of phase II PK analysis, will have PK blood draws at 10, 13, 16, 19 hrs post dose (8AM, 11AM, 2PM, 5PM +/- 10min).
- After completion of cycle 1 day 9 PKs, tucatinib should be dispensed
- Patient can resume tucatinib and continue letrozole and palbociclib per study schedule

7.2.4 Cycle 1 Day 15 (±2 days)

- Documentation of AEs
- Documentation of concomitant medications
- Vital signs (heart rate, BP, temperature), body weight
- Blood samples for hematology test (CBC and differential)
- PKs for palbociclib and tucatinib will be obtained in 20 subjects enrolled in phase Ib part of the study. PKs will be obtained prior to dosing of tucatinib and palbociclib, and in 0.5, 1, 2, 3, 4 and 6 hrs after the dose (according to PK schedule, section 8.3). Subject should take the morning dose of tucatinib and daily dose of palbociclib after pre-treatment PK blood draw has been obtained

7.2.5 Cycle 2 Day 1 and Day 1 of all subsequent cycles (±2 days)

- Documentation of AEs
- Documentation of concomitant medications
- Physical examination
- Vital signs (heart rate, BP, temperature), body weight
- ECOG PS
- Blood samples for hematology, clinical chemistry and liver function tests
- Blood samples for PK analysis prior to the dose of tucatinib and palbociclib – cycle 2 only, only for 20 subjects enrolled in phase Ib part (for full PK schedule, see section 8.3)
- Research blood sample for assessment of potential biomarkers of response (cycle 2 only)
- Review tucatinib, palbociclib and letrozole drug compliance from previous cycle
- Dispense tucatinib and palbociclib
- Administer the morning dose of tucatinib
- For patients enrolled in phase II part of the study that will have PK analysis on cycle 2 day 9, recommend to take palbociclib and letrozole at PM time (otherwise – palbociclib and letrozole can be taken at any time during the day as long as 24hr intervals between doses are maintained)

- Highly encouraged optional tumor biopsy (can be performed at any time during cycle 2).

7.2.6 Cycle 2 Day 8

- Patients who have PKs scheduled on the next day (day 9) should take high calorie / high protein meal at 8:00PM, and take tucatinib and palbociclib at 10:00PM. Phone call by coordinator is recommended to remind the patient about timing. Patient can take letrozole at any time during the day. Patient records timing of study drugs in the diary.

7.2.7 Cycle 2 Day 9

- Patients who are part of phase II PK analysis, will have PK blood draws at 10, 13, 16, 19 hrs post dose (8AM, 11AM, 2PM, 5PM).
- Patient will be due for morning dose of tucatinib approximately at 10AM and should take it about 10:00AM (between PK blood draws)
- After completion of PKs, all study medications are continued per study protocol

7.2.8 Cycle 2 Day 15 (±2 days)

- Documentation of AEs
- Documentation of concomitant medications
- Vital signs (heart rate, BP, temperature), body weight
- Blood samples for hematology (CBC and differential)

7.2.9 Cycle 2, 4, and 6, day 26 (±3 days)

- Imaging studies to document sites of disease and assess tumor burden based upon RECIST 1.1 and/or RANO-MB. Recommended imaging include CT chest, abdomen, and pelvis with contrast and NM bone scan. Additionally, contrast-enhanced brain MRI should be performed in subjects with known CNS metastatic disease.

If cycles are delayed for any reason, then perform radiographic assessment of all known sites of metastatic disease 8 weeks (±3 days) from the previous scan.

7.2.10 Cycle 3 day 26, and thereafter every third cycle (±3 days)

- Transthoracic ECHO or MUGA, using the same cardiac testing modality performed in screening / baseline

If cycles are delayed for any reason or there is an interim assessment, then perform 12 weeks (±3 days) from previous ECHO or MUGA.

7.2.11 Cycle 9 day 26, and thereafter every third cycle (±3 days)

- Imaging studies to document sites of disease and assess tumor burden based upon RECIST 1.1 and/or RANO-MB. Recommended imaging include CT chest, abdomen, and pelvis with contrast and NM bone scan. Additionally, contrast-enhanced brain MRI should be performed in subjects with known CNS metastatic disease.

If cycles are delayed for any reason or there is an interim assessment, then perform 12 weeks (±3 days) from the previous scan.

7.3 End of treatment visit

End of treatment visit will occur for all subjects when disease progression is documented and/or subsequent anticancer therapy is initiated, or the subject is withdrawn from treatment for any other reason. The visit should occur at the time or within 7 days after the subject discontinued study drugs. The following procedures/assessments will be carried out as described in the Study Calendar and unless performed in the previous week. Every effort should be made to have a final tumor assessment.

- Documentation of AEs
- Documentation of concomitant medications
- Review tucatinib, palbociclib and letrozole drug compliance from previous cycle
- Vital signs, including weight
- Physical exam
- ECOG performance status
- Hematology, blood chemistry, liver function tests
- Imaging studies to document sites of disease and assess tumor burden based upon RECIST 1.1 and/or RANO-MB. Recommended imaging include CT chest, abdomen, and pelvis with contrast and NM bone scan. Additionally, contrast-enhanced brain MRI should be performed in subjects with known CNS metastatic disease. If a subject had required imaging studies within 30 days from visit, these studies will not need to be repeated.
- ECHO or MUGA, as appropriate, if not done within previous 12 weeks
- Tumor biopsy (optional)
- Research blood sample

7.4 Thirty days post-treatment follow-up (±3 days)

Additional safety follow up visit shall occur in 30±3 days after the subject discontinued treatment with study drug. This visit shall include:

- Documentation of AEs
- Documentation of concomitant medications
- Vital signs, including weight
- Physical exam
- ECOG performance status
- Hematology, blood chemistry, liver function tests

7.5 Subject withdrawal

In the absence of treatment delays due to AE(s), treatment may continue until one of the following criteria applies:

- Disease progression
- Death
- Specific changes in the subject's condition that render the subject unacceptable for further treatment in the judgment of the investigator
- Subject non-compliance with protocol requirements (determined via meeting with PI or Sub-Investigator, clinical research coordinator, and subject)
- Unacceptable AE(s). Any grade 4 study-drug related AE is unacceptable and will lead to withdrawal

- Withdrawal of consent (attempts should be made to clarify with the subject, if withdrawal is specific to study participation, or if it applies to data collection as well). Note: subjects lost to follow up (defined as no contact > 42 days after last on-study contact and at least 3 attempts) are considered to have withdrawn study participation consent. Data collection is permissible. Date of last contact will be recorded as the withdrawal date.
- Study termination by sponsor

In case of intercurrent illness that prevents administration of the study drugs for ≥21 days, or other reasons for the interruption of therapy for ≥21 days unrelated to disease progression / study drug toxicity (for example, loss of insurance), consultation with the lead study PI is advised. Such rare cases should be considered on an individual basis (depending on subject's prior response to study therapies, stability of disease, and duration of treatment interruption). Decision whether a patient can go back on study in this situation should be discussed between the Investigator and the lead study PI.

The reason for withdrawal must be recorded in the subject's electronic case report form (eCRF). The subject should complete the evaluations scheduled for the End of Treatment Visit and Safety Follow-up, provided written consent to do so has not been withdrawn. If an unacceptable AE is the cause for withdrawal, then "Adverse Event" should be recorded as the reason for withdrawal rather than physician decision or subject decision. Subjects who discontinue tucatinib or both palbociclib and letrozole will be withdrawn from the study; reason for withdrawal for these subjects should be recorded as an "adverse event" if AE led to discontinuation of study drugs. Subjects who withdraw from the study will not be replaced.

8 ASSESSMENTS

8.1 Safety evaluations

Safety assessments will consist of monitoring and recording AEs and SAEs, physical examination and vital signs, and measurement of protocol-specified clinical laboratory tests, ECG, and imaging studies. Clinically significant changes in these parameters may be captured as AEs and SAEs. The investigator must notify Criterium and Pfizer Inc. of any SAE experienced by subject within 24 hours of first awareness of the event (immediately upon awareness if the event is fatal or life-threatening). Subsequent timely notification of the Lead PI, medical monitor, Seattle Genetics, Inc. and the University of Colorado Cancer Center DSMC will be done by Criterium. SAE reporting is described in detail in chapter 9.9.1.

The investigator is responsible for the appropriate medical care and the safety of subjects who have entered this study. The investigator must document all AEs and SAEs, including the type, severity (graded by NCI CTCAE version 4.03), seriousness, timing and attribution to study drugs. Baseline signs and symptoms will be recorded at the time of study enrollment and then reported as AEs during the trial if they worsen in severity or increase in frequency.

8.1.1 Laboratory assessments

All safety labs will be analyzed by the site's local laboratory. The minimum requested lab data includes:

- Hematology
- White blood cell count (WBC)
- Absolute neutrophil count (ANC)
- Platelet count

- Hemoglobin
- WBC differential

Blood Chemistry:

- Glucose
- BUN
- Creatinine
- Sodium
- Potassium
- Calcium

Liver function tests:

- Albumin
- ALT
- AST
- Alkaline phosphatase
- Total bilirubin

Coagulation tests:

- PT/INR
- PTT

Investigators may order additional blood tests for planning treatment administrations, dose modification, or further evaluation of AEs.

8.1.2 EKG assessments

Twelve lead EKGs will be performed at baseline. Electrocardiogram assessments are recommended to be performed after the measurement of vital signs, with the subject supine and rested for 5 minutes. The date and time of EKG assessment should be recorded.

8.1.3 Safety plan for cardiotoxicity

HER2-targeted therapies are known to increase the risk of the development of asymptomatic and symptomatic declines in LVEF. Cardiac function will therefore be monitored closely. Subjects will be monitored throughout the study for the occurrence of any cardiac symptoms. Assessment of cardiac ejection fraction will be performed by MUGA or ECHO at baseline screening, once every 12 weeks (±3 days) thereafter until study discontinuation, and at the end of treatment visit unless done within 12 weeks prior this visit. Whichever testing modality is chosen in screening should be used for all subsequent cardiac assessments throughout the study for comparison. Dose modifications or discontinuation of tucatinib in case of cardiac toxicity will be performed as described in the section 6.3.1. Any decline in LVEF leading to a change of tucatinib dose or discontinuation of tucatinib treatment is considered an event of special interest and an SAE, and must be reported within 24 hours of discovery of the event as described in the sections 9.9.1 and 9.9.3.

8.1.4 Safety plan for hepatotoxicity

While not among the most common adverse reactions reported in subjects taking tucatinib, grade 3 and 4 elevation of LFTs has been seen in some subjects on tucatinib, and monitoring of liver function tests is required for any subject taking tucatinib. LFT elevations seen in the studies of tucatinib are briefly summarized below (data cut-off date of 27 May 2015).

Among 41 subjects treated in the Phase 1b combination study of tucatinib plus capecitabine and trastuzumab (Study ONT-380-005), only 2 subjects (5%) experienced grade 3 elevation of AST/ALT, and in both subjects the elevation was reversible and the subjects were able to continue on study after dose reduction. Of the 2 subjects with grade 3 elevation of AST/ALT, one was treated with tucatinib plus capecitabine, while the other was treated with tucatinib plus capecitabine plus trastuzumab. In the Phase 1b study of tucatinib plus TDM-1 (Study ONT-380-004), elevations of liver function tests were observed more frequently than in Study ONT-380-005. This is likely due to the fact that this is an overlapping toxicity with TDM-1, which has previously been associated with hepatotoxicity. Grade 3 AST/ALT elevation occurred in 7 out of 43 subjects (16%) and has been reversible with dose interruption and reduction except in 2 subjects found to have progressive liver metastases. An additional subject in the Dana Farber Cancer Institute investigator-sponsored trial had an event of grade 4 hepatocellular toxicity, with grade 4 elevation of ALT/AST and grade 3 elevation of total bilirubin. The subject's LFTs normalized after discontinuation of ONT-380. Complicating features of the event included the concomitant use of trimethoprim/sulfamethoxazole, which may increase tucatinib levels via inhibition of CYP2C8. In all the studies of tucatinib to date there were no cases of LFT elevation meeting the protocol pre-determined criteria for drug-induced liver injury [74].

Because of the known risk of elevation of liver enzymes with tucatinib, subjects will have LFTs closely monitored. Any potential case of drug-induced liver injury (Hy's law) will be treated as a protocol-defined event of special interest and an SAE if it meets the following criteria: AST or ALT elevations that are > 3 X ULN with concurrent elevation of total bilirubin > 2 X the ULN (within 21 days of AST and/or ALT elevations), except in subjects with documented Gilbert's syndrome [74]. Any such events should be promptly reported within 24hours of discovery as described in the sections 9.9.1 and 9.9.3. Tucatinib drug hold and dose modifications for LFT elevations not meeting these criteria are outlined in in section 6.3.1.

Although LFT elevation is much more likely to be attributed to tucatinib, palbociclib may contribute. Therefore, **in all cases of bilirubin or ALT / AST elevation of≥G3, both tucatinib and palbociclib should be put on hold, and lead PI should be consulted regarding the attribution of toxicity.**

8.1.5 Safety plan for neutropenia and infection risk

Decreased neutrophil count and infections are common side effects of palbociclib, although in all published studies of palbociclib to serious infections in the settings of neutropenia were relatively uncommon. Pooled analysis of PALOMA-1 and PALOMA-2 clinical trials demonstrated that grade 3 and 4 neutropenia was reported in 55.3 and 10.1%, respectively, in subjects receiving palbociclib plus letrozole [75]. In PALOMA-1 clinical trial, median time to the first episode of any grade neutropenia was 15 days (13-117 days), median duration of Grade ≥3 neutropenia was 7 days. WBC and ANC will therefore be monitored closely in all subjects enrolled into this clinical trial. Subjects will have WBC and ANC measured prior to starting palbociclib therapy, at the beginning of each cycle, on Day 15 of the first two cycles, and as clinically indicated. Dose modifications or discontinuation of palbociclib in case of severe neutropenia will be performed as described in Section 6.3.2. In the current protocol, dose modification plan for palbociclib follows FDA recommendations listed in the palbociclib Full Prescribing Information Brochure [28].

Infections have been reported at a higher rate in subjects treated with palbociclib plus letrozole compared to subjects treated with letrozole alone: Grade 3 or 4 infections occurred in

5.2% of subjects treated with palbociclib plus letrozole whereas only 2.5% of subjects treated with letrozole alone experienced a Grade 3 infection, and no one experienced a Grade 4 infection [75]. Therefore, study subjects will be closely monitored for signs and symptoms of infections and treated with antibiotics as medically appropriate. All serious neutropenic and infectious AEs will be promptly reported as described in section 9.9.1.

8.1.6 Safety plan for subjects with CNS metastases

Subjects with CNS metastases are at risk for occurrence of AEs due to the presence of CNS lesions, progression of disease and toxicities potentially related to study treatment. In order to minimize the risk of AEs in subjects with brain metastases in this study, subjects with high-risk metastases, including those requiring immediate local therapy, those with rapidly progressing lesions, those requiring unstable doses of corticosteroids at the start of the study for control of CNS symptoms are excluded from the trial. Enrollment of subjects with asymptomatic untreated CNS lesions >2cm is discouraged, but may be allowed after a consultation with Lead PI.

On occasion, treatment of CNS metastases with systemic or radiation therapy has been associated with localized edema thought to be due to treatment effect and not tumor progression. Under select circumstances, as outlined in the RANO-BM criteria, subjects may receive corticosteroid therapy for acute management of symptomatic local edema, as long as brain MRI does not show clear evidence of CNS progression. All such instances require approval from the Lead PI.

Because of previously described association between approved HER2-targeted therapies and rare cases of brain edema [76], any event of cerebral edema not clearly attributable to progression of disease should be reported as an event of special interest within 72 hours. Grade 3 cerebral edema meeting criteria for an SAE should be reported within 24 hours, as described in the sections 9.9.1 and 9.9.3.

8.1.7 Safety plan for prevention of pregnancy

Due to the potential effect on embryo-fetal development from all three drugs used in this study, premenopausal women of childbearing potential defined as premenopausal women who are not permanently sterile (i.e., due to hysterectomy, bilateral oophorectomy, bilateral tubal ligation, or bilateral tubal occlusion) are required to have negative pregnancy tests prior to initiation of treatment. Women of childbearing potential and men with partners of childbearing potential must agree to use a highly effective form of non-hormonal contraception by the patient and/or partner. Hormonal contraception is contraindicated in patients with HR+ breast cancer, therefore, it cannot be used by study subjects. Effective methods of non-hormonal contraception include IUD; female condom; diaphragm with spermicide; cervical cap; use of a condom by the sexual partner; or a sterile sexual partner. Women of childbearing potential and men with partners of childbearing potential enrolled in this study should practice effective method of contraception, as described above, starting from the signing of informed consent until 30 days after the end of the study.

8.2 Efficacy evaluations

Following initiation of study treatment, measurement of all known sites of metastatic disease will be obtained at the end of every 8 week period (±3 days) for study cycles 1-6, then at the end of every 12 week period (±3 days) until PD, initiation of a new therapy, withdrawal of consent, or study closure. Recommended imaging include contrast-enhanced CT of the chest, abdomen and pelvis and NM bone scan. For subjects with known CNS disease, restaging imaging studies must

include contrast-enhanced MRI of the brain. Detailed schedule of radiographic assessments is specified in the section 7.2 of the protocol. The scans will remain on this schedule irrespective of dose interruptions. The same imaging technique used to characterize each identified and reported lesion at baseline will be employed in the following tumor assessments. Additional imaging may be done as appropriate at the discretion of the investigator.

All subjects' files and radiologic images must be available for source verification if required. All treatment decisions will be made on the basis of local investigator assessment of radiologic imaging based on RECIST version 1.1, or, for subjects with CNS disease, based on RECIST 1.1 and RANO-BM (as defined in the Appendix C).

In the event of equivocal progression and no imminent threat to subject safety, all efforts should be made to continue the subject until unequivocal radiologic or clinical progression is documented, as defined in RECIST 1.1 and/or RANO-BM.

In this trial, if a subject is found to have progressive disease due to CNS progression per RANO-BM, but does not have progressive disease outside the CNS per RECIST 1.1, the subject may undergo local therapy (radiotherapy or surgery) to the progressive CNS lesion(s), and continue on study after discussion with and approval from the PI. The subject may continue on study until a second progressive disease event occurs (either CNS or non-CNS).

8.3 Pharmacokinetic evaluations

PK assessments of blood levels of tucatinib and palbociclib will be performed on Cycle 1 Day 15 and Cycle 2 Day 1 of therapy in 20 participants enrolled in phase Ib part of the study. Plasma samples will be collected to measure levels of tucatinib and its hydroxyl metabolite, as well as levels of palbociclib and, if needed, its metabolites at steady state on Cycle 1 Day 15. Plasma samples will also be collected prior to administration of tucatinib and palbociclib on the first day of Cycles 2 to assess trough levels.

Additional PK assessment will be done in 5 to 10 patients enrolled in phase II part of the study to evaluate the levels of tucatinib and palbociclib. These PKs will be done on Cycle 1 Day 9 and Cycle 2 Day 9. Prior to the first set of PKs, on Cycle 1 Days 1-8, patient will take palbociclib and letrozole; tucatinib will be on hold. Tucatinib should be dispensed and started per usual study schedule after the first set of PKs is obtained on Day 9. Prior to the second set of PKs, on Cycle 2 Days 1-8, patient will take palbociclib, letrozole and tucatinib (all study drugs) per usual study schedule. On the day prior to PK evaluation (Day 8 of Cycle 1 and Day 8 of Cycle 2), patient should consume high calorie / high fat meal at 8:00PM and take study drugs at 10:00PM. On the next day, plasma samples will be collected to measure PKs at 10, 13, 16 and 19 hrs +/- 10 minutes post dose (8:00AM, 11:00AM, 2:00PM and 5:00PM on Cycle 1 Day 9 and Cycle 2 Day 9). For detailed information on phase II PKs, refer to the LabConnect Manual (PK collection manual) provided by Seattle Genetics, Inc.

Table 19. Schedule of pharmacokinetic assessments

Cycle	Day	Time point
Phase Ib PK assessment		
1	15	0 h (pre-dose) prior to dosing of tucatinib and palbociclib
		0.5 h (±10 minutes) following dosing of tucatinib and palbociclib
		1 h (±10 minutes) following dosing of tucatinib and palbociclib
		2 h (±10 minutes) following dosing of tucatinib and palbociclib

		3 h (±10 minutes) following dosing of tucatinib and palbociclib
		4 h (±10 minutes) following dosing of tucatinib and palbociclib
		6 h (±30 minutes) following dosing of tucatinib and palbociclib
2	1	0 h (pre-dose) prior to dosing of tucatinib and palbociclib
Phase II PK assessment (late PK time points)		
1	1-8	Patient takes palbociclib 75mg and letrozole 2.5mg PO every evening (tucatinib is on hold). On day 8, patient consumes high fat / high calorie meal at 8:00PM and take palbociclib at 10:00PM (leterozole at any time). Patient record time of study medications in the diary.
1	9	10 h (±10 minutes) following dosing of palbociclib (8AM)
		13 h (±10 minutes) following dosing of palbociclib (11AM)
		16 h (±10 minutes) following dosing of palbociclib (2PM)
		19 h (±10 minutes) following dosing of palbociclib (5PM)
1	9-28	Post-PKs, patient starts tucatinib, continues on palbociclib and letrozole per study schedule
2	1-8	Patient takes palbociclib, tucatinib and letrozole per study schedule. On day 8, patient consumes high fat / high calorie meal at 8:00PM and take palbociclib and the evening dose of tucatinib at 10:00PM (leterozole at any time). Patient record time of study medications in the diary.
2	9	10 h (±10 minutes) following dosing of tucatinib and palbociclib (8AM)*
		13 h (±10 minutes) following dosing of tucatinib and palbociclib (11AM)
		16 h (±10 minutes) following dosing of tucatinib and palbociclib (2PM)
		19 h (±10 minutes) following dosing of tucatinib and palbociclib (5PM)

*About 10:00AM patient will be due for the next morning dose of tucatinib, and should take it approximately at this time

All efforts will be made to obtain the PK samples at the exact nominal time relative to dosing. However, samples obtained within 10 minutes of the nominal time is acceptable, with the exact time of the sample collection noted on the source document and CRF. PK samples will be assayed with liquid chromatography. For further details on collection, processing and shipment of PK samples, see LabConnect manual.

In addition to the samples collected at the scheduled times, additional PK blood samples may be requested from subjects experiencing unexpected AEs or SAEs.

8.4 Collection of patient material for correlative studies

Subjects will have three research blood samples drawn pre-treatment, after 28 days of treatment (±3 days), and after completion of all study treatments at the end of the study. These blood samples will be obtained for ctDNA analysis and analysis of other circulating markers associated with cancer prognosis or outcomes from treatment. Subjects will be asked to undergo tumor core needle biopsies pre-treatment and at any time during cycle 2. These paired tumor biopsies are optional, but highly encouraged. For highly motivated subjects there will be an option to get a third tumor biopsy at the time of disease progression or study withdrawal for other reasons. For detailed instructions on collection, processing and shipment of research blood samples and biopsy samples, please refer to the Study Lab Manual.

Residual blood and tumor samples left after analysis may be saved in the study biorepository if study subject provides voluntary consent for future research.

8.5 Concomitant medications

Medications used by the subject and therapeutic procedures completed by the study subject will be recorded in the CRFs from screening through 30 days after the last dose of study drug.

9 ADVERSE EVENT REPORTING

9.1 Adverse events

All observed or volunteered AEs regardless of suspected causal relationship to the study drugs will be reported as described in the following sections. For all adverse events, the investigator must pursue and obtain information adequate both to determine the outcome of the AE and to assess whether it meets criteria for classification as SAE (see Section 9.5). The investigator is required to assess causality. For SAEs with a causal relationship to the study drugs, follow-up by the investigator with subsequent submission of SAE follow up report (as described in section 9.9.1) is required until the event or its sequelae resolve or stabilize at a level acceptable to the investigator.

9.2 Reporting period

Reporting period begins from the day the subject signs the consent form through 30 calendar days after the last administration of the study drugs. During this time all AEs should be recorded in the CRFs. Any SAE occurring any time during the reporting period must be promptly reported to Criterium and Pfizer, Inc. within 24 hours of Investigator learning about an SAE (immediately upon awareness if the event is fatal or life threatening). Subsequent timely notification of the Lead PI, medical monitor, Seattle Genetics, Inc. and University of Colorado Cancer Center DSMC will be done by Criterium. Lead study PI will report all SAEs to the Colorado Multiple Institutional Review Board (COMIRB) as directed by COMIRB policy.

Additionally, Investigator should report SAEs to the local DSMC or DSMB at his / her institution per local DSMC/DSMB policy, and to the institutional IRB per local IRB policy.

If Investigator becomes aware of an SAE occurring any time after the administration of the last dose of the study drugs, Investigator should report that SAE if he/she suspects a causal relationship between the study drugs and an SAE.

9.3 Definition of an Adverse Event

An AE is any untoward medical occurrence in a clinical investigation subject administered a product or medical device; the event need not necessarily have a causal relationship with the treatment or usage. Examples of AEs include but are not limited to:

- Abnormal test findings
- Clinically significant symptoms and signs
- Changes in physical examination findings
- Hypersensitivity

Additionally, they may include the signs or symptoms resulting from:

- Drug overdose
- Drug withdrawal
- Drug abuse
- Drug misuse
- Drug interactions
- Drug dependency

Worsening signs and symptoms of the malignancy under trial should be reported as AEs in the appropriate section of the CRF. Disease progression assessed by measurement of malignant lesions on radiographs or other methods should not be reported as an AE unless the outcome is fatal within the safety reporting period.

9.4 Abnormal Test Findings

The criteria for determining whether an abnormal objective test finding should be reported as an AE are as follows:

- Test result is associated with accompanying symptoms, and/or
- Test result requires additional diagnostic testing or medical/surgical intervention, and/or
- Test result leads to a change in study drug dosing or discontinuation of the trial, significant additional concomitant drug treatment, or other therapy, and/or
- Test result is considered to be an AE by the investigator or sponsor.

Merely repeating an abnormal test, in the absence of any of the above conditions, does not constitute an AE. Any abnormal test result that is determined to be an error does not require reporting as an AE.

9.5 Serious Adverse Events

An SAE is any event, without regard to causality, that is life-threatening (i.e., causes an immediate risk of death) or that results in any of the following outcomes: death; in-patient hospitalization or prolongation of existing hospitalization; persistent or significant disability or incapacity (i.e., substantial disruption of the ability to conduct normal life functions); or a congenital anomaly or birth defect. Any other medical event that, in the medical judgment of the Investigator, may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed above is also considered an SAE. A planned medical or surgical procedure is not, in itself, an SAE.

Cases of potential drug-induced liver injury as assessed by laboratory test values and outlined in section 8.1.4 meet definition of SAE. If a study subject develops abnormal values of AST or ALT or both concurrent with abnormal elevation of total bilirubin and no other known cause of liver injury (ALT or AST >= 3x ULN and total bilirubin >= 2x ULN) [74] as defined in section 8.1.4), this event should be reported as SAE.

Progression of the malignancy under trial (including signs and symptoms of progression) is reported as SAE only if it resulted in hospitalization, or if the outcome is fatal within the safety reporting period. If the malignancy has a fatal outcome during the trial or within the safety reporting period, then the event leading to death must be recorded as an adverse event and as SAE with CTCAE grade 5 (see Section 9.7, Severity Assessment).

9.6 Hospitalization

Adverse events reported from clinical trials associated with hospitalization or prolongation of hospitalization is considered serious. Hospitalization does not include the following:

- Rehabilitation facilities
- Hospice facilities
- Respite care
- Skilled nursing facilities
- Nursing homes
- Same day surgeries (out-patient/same day/ambulatory procedures)

Hospitalization or prolongation of hospitalization in the absence of a precipitating, clinical AE is not in itself a SAE. Examples include:

- Admission for treatment of a preexisting condition not associated with the development of a new AE, or with a worsening of the preexisting condition clearly not related to the study drugs
- Social admission (e.g., patient has no place to sleep)
- Admission for a procedure required by the trial protocol
- Optional admission not associated with a precipitating clinical AE (e.g., for elective cosmetic surgery)
- Hospitalization for observation without a medical AE
- Admission exclusively for the administration of blood products

9.7 Severity Assessment

The investigator will use the definitions of Severity in accordance with NCI-CTCAE version 4.03 to describe the AE.

Table 20. General AE Grading Assessment

GRADE	Clinical Description of Severity
1	**MILD** adverse event
2	**MODERATE** adverse events
3	**SEVERE** adverse events
4	**LIFE-THREATENING OR DISABLING** adverse events
5	**DEATH RELATED TO** adverse events

Note the distinction between the severity and the seriousness of an AE. A severe event is not necessarily a serious event. For example, a headache may be severe (interferes significantly with patient's usual function) but would not be classified as serious unless it met one of the criteria for SAEs, listed above.

9.8 Causality Assessment

The investigator's assessment of causality must be provided for all AEs (serious and non-serious). An investigator's causality assessment is the determination of whether there exists a reasonable possibility that the investigational product caused or contributed to an adverse event. If the investigator's final determination of causality is unknown and the investigator does not know whether or not investigational product caused the event, then the event will be handled as "related to investigational product" for reporting purposes.

9.9 Reporting Requirements

Each AE is to be assessed to determine if it meets the criteria for an SAE, and reported promptly according to requirements specified below. All AEs will be reported on the AE page(s) of the CRF. Additionally, investigators will report SAEs using separate SAE reporting forms. The reportable event fax cover sheet must be included with each SAE submitted.

9.9.1 Serious adverse event reporting requirements

All AEs that are serious, regardless of relationship to study drug, that occur after the subject signs the consent form must be reported to Criterium and Pfizer, Inc. on a SAE form with the reportable event fax cover sheet within 24 hours of discovery of the event (immediately if event is fatal or life threatening). SAE form and reportable event fax cover sheet should be faxed to Pfizer, Inc. (**Pfizer U.S. Clinical Trial Department fax #: 1-866-997-8322**). The SAE report form and reportable event fax cover sheet should be scanned and e-mailed to Criterium (**ABRCCPfizerSAE@criteriuminc.com**). Upon receiving information about SAE, Criterium will forward this information to the Lead PI, medical monitor, Seattle Genetics, Inc. and the University of Colorado Cancer Center DSMC in a timely manner. Additionally, initial and follow up SAE reports should be provided by site investigators to their IRBs and DSMCs/DSMBs in accordance with their policies. Lead study PI will submit information on all SAEs to the COMIRB in accordance with the COMIRB policy. Any new information or follow-up information pertaining to previously reported SAEs should be reported within 24 hours of becoming aware of the new or follow-up information. For initial SAE reports, at a minimum, the following should be included:

- Subject study ID number
- AE term(s), including serious criteria and onset date
- Study treatment
- Causality assessment

Investigators should report SAEs as soon as they are determined to meet the definition, even if complete information is not yet available. In the rare event that the investigator does not become aware of the occurrence of SAE immediately (e.g., if a subject initially seeks treatment elsewhere), the investigator is to report the event within 24 hours after learning of it, and document the time of his/her first awareness of the SAE.

All information about SAE should be promptly entered by study coordinators in the study database on AE pages of eCRFs. Source verification of the database AE information against SAE report forms will be performed by Criterium.

An investigator may be requested by the Lead PI, medical monitor, Pfizer Inc., Seattle Genetics, Inc., or Criterium to obtain specific additional follow-up information in an expedited fashion. This information collected for SAEs is more detailed than that in the SAE form or in the AE pages in the CRFs. In general, this will include a description of the SAE in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. In the case of a subject death, a summary of autopsy findings (if available) must be submitted as soon as possible. Study investigators and Lead PI will assist Pfizer Inc. and Seattle Genetics, Inc. in investigating any SAE and will provide follow-up information reasonably requested by Pfizer Inc. and Seattle Genetics, Inc..

The SAEs that are subject to this reporting provision are those that occur from the day subject signs the informed consent form through 30 calendar days after the last administration of the study drugs. In addition, if an investigator becomes aware of an SAE occurring any time after the administration of the last dose of the study drugs, the investigator should report that SAE if he/she suspects a causal relationship between the drug products and the SAE.

9.9.2 Non-serious adverse event reporting requirements

Non-SAEs are to be reported on the AE page of the CRF.

9.9.3 Events of special interest

Several types of SAEs represent events of special interest on this clinical trial:

- Left ventricular dysfunction or symptomatic CHF leading to a change of tucatinib dose or discontinuation of tucatinib treatment (as outlined in Section 8.1.3)
- Liver toxicity meeting Hy's law definition (as outlined in Section 8.1.4)
- Any cerebral edema not clearly attributable to progression of disease (as outlined in Section 8.1.6)

Left ventricular dysfunction or symptomatic CHF leading to a dose modification or discontinuation of tucatinib, liver toxicity meeting Hy's law and grade 3 cerebral edema should follow SAE reporting requirements and be reported within 24hrs from the discovery of event. Grade 1 and 2 cerebral edema not clearly attributable to progression of disease must be reported within 72 hrs of the discovery of event.

9.9.4 Pregnancy reporting requirements

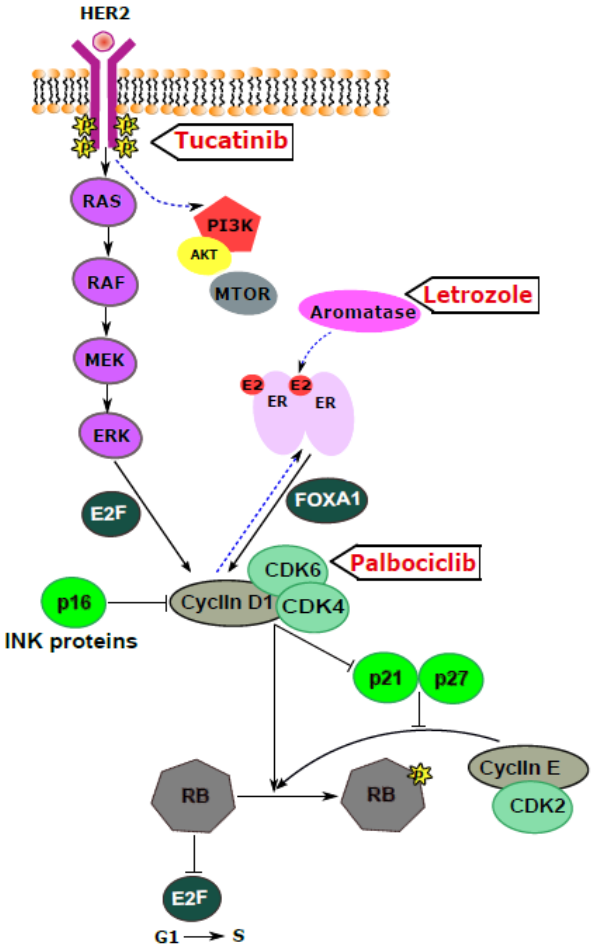
If a study participant becomes pregnant during administration of the drug, all study treatments must be discontinued. The investigator should report all pregnancies within 24 hours on the Pregnancy Reporting Form per the SAE reporting guidelines (section 9.9.1). The investigator will be asked for a follow up evaluation of the pregnancy, fetus, and child. Abortion, whether accidental, therapeutic, or spontaneous, should be reported as a SAE. Congenital anomaly or birth defects should also be reported as a SAE. All pregnancies will be monitored for the full duration; all perinatal outcomes should be reported. Infants should be followed for a minimum of 8 weeks and all neonatal outcomes should be reported.

10 CORRELATIVE STUDIES

10.1 Background and rationale

The study incorporates a correlative biomarker component using paired pre- and post-treatment biopsy samples, as well as liquid biopsy, to explore sensitivity of tumors to triple combination therapy and putative resistance mechanisms potentially targetable with future drug combinations.

Correlative studies will be centered on HER2, ERα and RB (cell cycle checkpoints) pathways (Fig. 3). HER2/MAPK kinase signaling activates E2F transcription factors leading to transcription of cyclin D1 gene. Active ERα in complex with FOXA1 transcription factor amplifies cyclin D1 transcription through an estradiol (E2)-responsive enhancer. Cyclin D1 couples with CDK4/6 and phosphorylates RB. P16 and other INK-family proteins inhibit cyclin D1 – CDK4/6 complex, however, p16 is frequently inactivated in breast tumors [67]. Cyclin D1 increases transcriptional activity of ERα, providing a positive feedback loop. In addition to being catalytically active, the cyclin D1 – CDK4/6 complex sequesters the cell-cycle inhibitors p21 and p27, therefore promoting the activation of the cyclin E – CDK2 complex further phosphorylating RB. Hyperphosphorylated RB loses its inhibitory effect on the E2F transcription program, allowing G1 to S transition [77, 78]. Because cyclin E itself is an E2F target gene, cyclin E may reinforce its own expression. Once cyclin E – CDK2 becomes active, RB phosphorylation is rendered partially independent of the mitogenic control that regulates cyclin D1 expression [79]. CDK2 phosphorylates ERα, further increasing ERα transcriptional activation [80].



10.2 Potential mechanisms of resistance and sensitivity to tucatinib

In addition to cyclin E overexpression, an important mechanism of resistance to HER2-targeted agents is activation of PI3K/AKT signaling, mediated by HER2 preferential dimerization partner HER3 [81]. Inhibition of HER2 by all currently approved HER2-targeted agents induces a robust increase in HER3 expression [81]. The HER3 protein tail has six PI3K-binding motifs, more than most kinases, and activation of HER3 leads to a robust PI3K pathway activation. Additionally, genetic or epigenetic events in PI3K subunits, PTEN or AKT may lead to the deregulation of this pathway and escape from HER2-blockade [81-83].

Little is known about *HER2* gene mutations mediating resistance to HER2-targeted agents. The frequency of *HER2* mutations in early stage HR+/HER2+ breast cancer is 4% (TCGA analysis March 26, 2016), however, the frequency of *HER2* mutations in HR+/HER2+ metastatic disease is unknown. Several potentially important genetic alterations of *HER2* have been described in pre-clinical studies. L755S mutation produces lapatinib resistance. In-frame deletion of nucleotides

755-759 in the *HER2* gene, which is homologous to EGF receptor (*EGFR*) exon 19 in-frame deletions, leads to increased phosphorylation of EGFR or HER3 [84].

10.3 Potential mechanisms of resistance and sensitivity to palbociclib

Resistance to CDK4/6 inhibition may be mediated by overexpression of cyclin E – CDK2 [85], which is also a mechanism of resistance to HER2 inhibitors [85] and letrozole [86]. Cyclin E overexpression occurs in 18-22% of breast carcinomas [87] and results from mutations in mitogenic pathways upstream of cyclin E [79], cyclin E gene (*CCNE*) amplification [88], or disrupted cyclin E ubiquitination and proteolysis [79, 89] mediated by Fbw7 tumor suppressor protein. Fbw7 inactivation could contribute to cyclin E overexpression. The prevalence of *Fbw7* gene mutations in breast tumors is not known, although it is recognized that loss of chromosome 4q32 containing *Fbw7* gene occurs in up to 30% of solid tumors [89].

Potential markers of sensitivity to CDK 4/6 inhibitors may include p16 inactivation, indicating tumor addiction to the RB pathway, and the presence of normal RB expression at baseline [20]. Tumors with RB loss are utilizing tumorigenic pathways other than cell cycle checkpoints.

10.4 Potential mechanisms of resistance and sensitivity to letrozole

Many known mechanisms of letrozole resistance are linked to the activation of the RB pathway including cyclin D1 amplification or overexpression [90], activating mutations in *CDK4* gene, RB loss, p21 and p27 loss or abnormal cellular compartmentalization [90-92]. These mechanisms of resistance will be counteracted by co-administration of palbociclib. Upregulation of the HER2 pathway leads to endocrine resistance [93], however, this will be addressed by treatment with tucatinib. The remaining potential resistance mechanisms include cyclin E and C-MYC overexpression [86, 90]. Besides, *ESR1* mutations associated with constitutive ligand-independent transcriptional activity of ERα (such as mutations in *ESR1* ligand binding domain at positions L536, Y537, D351, D538), may lead to resistance to anti-hormonal therapy [94-96].

10.5 Assessment of potential biomarkers of sensitivity to the study drugs

Summary of the potential mechanisms of resistance and sensitivity to the study drugs is presented in Table 21.

Table 21. Potential biomarkers of resistance and sensitivity to the study drugs

Drug	DNA level	RNA / protein level
Palbociclib	*Fwb7* mutations or LOH *CCNE* amplification *CDK2* amplification	Cyclin E overexpression RB loss P16 loss*
Tucatinib	*HER2* mutations (L755S) *PTEN* mutations *AKT* mutations *PI3K* mutations *CCNE* amplification *CDK2* amplification	Cyclin E overexpression HER3 overexpression PI3K/AKT/MTOR pathway activation through overexpression / increased phosphorylation
Letrozole	*ESR1* mutations (L536, Y537, D351, D538) *CCNE* amplification *CDK2* amplification	Cyclin E overexpression C-MYC overexpression

*p16 loss is a potential biomarker of sensitivity, all other alterations are potential biomarkers of resistance

In the light of the above, we will plan to assess the most important potential biomarkers of resistance to combination therapy by IHC in FFPE tumor blocks obtained before treatment, on treatment and post-progression: cyclin E and CDK2 overexpression that could mediate resistance to all three targeted agents, RB loss (mediates resistance to palbociclib), p16 loss (potential marker of sensitivity to palbociclib), expression of HER3, AKT and phospho-AKT (markers of PI3K/AKT pathway activation associated with resistance to HER2-targeted agents). Additionally, PAM50 tumor subtypes may be assessed and the results correlated with the efficacy of treatment. Other markers will be considered based on results and any advances in the field.

Then we will apply a much broader approach to tumor expression profiling and mutational analysis, and perform mRNA sequencing with the goal to provide an unbiased approach and to capture both known and novel features. RNA expression and mutational profiles will be compared to reference normal values, then comparison will be performed in tumor biopsy pairs before and after treatment, and in responders versus non-responders. Pathway analysis will be performed, with particular attention to known alterations in ERα, HER2 / HER3 / MAPK, RB and PI3K pathways potentially associated with resistance to palbociclib, HER2 tyrosine kinase inhibitors and endocrine therapy.

While tumor sequencing is very useful in studying tumorigenesis and therapeutic resistance, subjecting subjects to multiple invasive procedures could be a challenge, and ctDNA analysis, as well as analysis of other potential biomarkers (such as circulating tumor cells, exosomes, etc) by liquid biopsy represents an attractive alternative. Tumor ctDNA is fragmented to about 150 bp and present in only a few thousand copies per milliliter of blood. However, it is feasible to detect known mutations present at about 2% mutant allele frequency directly from plasma with sensitivity and specificity of more than 97% [97]. Therefore, we will perform exploratory analysis of *ESR1* mutations (L536, Y537, D351, and D538) associated with endocrine resistance, and *HER2* mutation L755S associated with resistance to HER tyrosine kinase inhibitors and other pertinent markers for resistance in ctDNA extracted from serum blood samples of study subjects before therapy, on therapy, and post progression. If specific resistance mutations are identified in tumor biopsies by RNA sequencing, ctDNA samples may be analyzed for the presence of these mutations. Additional analysis of other circulating markers potentially associated with cancer prognosis or outcomes from treatment (such as, for example, circulating tumor cells and exosomes) may be performed.

Correlative studies may be modified to include other markers of interest as determined by novel publications and discoveries at the time when all biopsy and blood samples are collected. Correlative studies may be performed with various external institutions. For details on research blood and tissue collection, processing and shipment please see the Study Lab Manual.

11 DATA ANALYSIS AND STATISTICAL METHODS

11.1 Analytic plan for phase IB part of the study

This is a multicenter, open-label, single arm study to determine safety and efficacy of the tucatinib in combination with palbociclib and letrozole in post-menopausal subjects with HR+/HER2+ locally advanced unresectable or metastatic breast cancer.

11.1.1 Analytic plan for primary endpoint

The primary endpoint of the phase Ib part of this trial is safety and tolerability of combination therapy as evaluated by standard NCI CTCAE version 4.03. Interim safety analysis will be performed after 10 subjects are accrued to the Phase Ib and again after 20 subjects are accrued to the phase Ib prior to the initiation of phase II part. At these time, we will assess the proportion of subjects experiencing DLTs due to tucatinib and/or palbociclib as outlined in the section 4.2 "Safety analysis of the combination therapy" and below.

Incidence, nature and severity of all adverse events (AEs) that occur on therapy will be analyzed. Safety will be considered clinically meaningfully altered if 60% or greater proportion of subjects had DLTs attributable to palbociclib, or 20% or greater proportion of subjects had DLTs attributable to tucatinib, or 50% or greater proportion of subjects had DLTs that could be potentially attributable to both drugs (Table 22).

Table 22. Baseline toxicity rates and safety boundaries for palbociclib and tucatinib

Drug	Confidence level	N*	Baseline toxicity rate	Safety boundary rate	Upper limit CI	n**
palbociclib	0.9	10	0.45	0.6	0.812	8 out of 10
		20	0.45	0.6	0.751	15 out of 20
tucatinib	0.9	10	0.10	0.2	0.408	4 out of 10
		20	0.10	0.2	0.347	7 out of 20
both drugs	0.9	10	ND***	0.5	0.760	8 out of 10
		20	ND	0.5	0.694	14 out of 20

*N – total number of subjects; **n – number of subjects that had DLTs attributable to palbociclib and/or tucatinib that is set as safety boundary; ***ND – not determined

To determine the safety of the triple combination, we will plan to enroll 10 subjects at the baseline starting doses of tucatinib 300mg PO BID, palbociclib 125mg PO daily 21 days on and 7 days off, and letrozole 2.5mg PO daily on a 28 day cycle length (DL1). The safety monitoring plan and determination of any required dose de-escalations are outlined in section 4.2.

In addition to the safety analysis, descriptive statistics will be used to summarize all subject characteristics and treatment administration/compliance. Safety will be evaluated through summaries of AEs, AEs will be classified by system organ class (SOC) and preferred term using the Medical Dictionary for Regulatory Activities (MedDRA version 18.0); AE severities will be classified using the NCI CTCAE criteria version 4.03. In the event of multiple occurrences of the same AE with the same preferred term in one subject, the AE will be counted once as the occurrence.

Collected AE data will be summarized in the following categories:

- All AEs (regardless of grade)
- All Grade 3/4/5 AEs
- All drug-related AEs (regardless of grade)
- All AEs leading to study drug or study discontinuations
- All AEs of special interest (regardless of grade). These include LFT abnormalities, changes in cardiac ejection fraction, incidence of neutropenia and infections, and cerebral edema.

Additional data on extend of exposure, tolerability and compliance that aid in safety assessment will be collected:

- Extend of exposure will be assessed by analyzing study drug administration data, any dose modifications or dose holdings will be flagged. For each of the regimen components (tucatinib, palbociclib and letrozole), duration of exposure and number of treatment cycles will be summarized.
- For each of the regimen components (tucatinib, palbociclib, letrozole), tolerability will be assessed by tabulating the frequency of study drug discontinuations.
- For each of the regimen components (tucatinib, palbociclib, letrozole), treatment compliance (percent of actual to planned dosing) will be summarized.
- Additionally, subjects who discontinue study drug prematurely or withdraw from the study will be summarized and listed, with the reason for early termination and/or withdrawal. Major protocol violations will be summarized. All non-protocol specified anti-cancer therapies will be listed and summarized.

11.1.2 Analytic plan for secondary endpoints

As the primary purpose of phase IB part of the study is to evaluate safety of the combination therapy, no specific statistical hypotheses testing in regard to secondary end-points (PK analysis and preliminary efficacy analysis) is planned.

All subjects enrolled in phase IB part who complete at least one day of PK blood sampling will be included in the PK analyses. Plasma concentration/time data of the drugs obtained at the designated times will be summarized descriptively and presented graphically by dose and day of assessment. The concentration/time data will be analyzed using non-compartmental methods to obtain PK parameters in individual subjects. These PK parameters include the maximum plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}), area under the plasma concentration versus time curve to 24 hours (AUC_{24hr}) and area under the plasma concentration versus time curve to the time of the last measurable concentration (AUC_{last}). If data permits, area under the plasma concentration versus time curve to infinity (AUC_{inf}), terminal elimination half-life ($t_{1/2}$), oral plasma clearance (CL/F) and apparent volume of distribution (Vd/F) will be also estimated. Individual values and descriptive statistics of these PK parameters will be provided by dose and day of assessment in tabular form. Exploratory analyses investigating the relationship between computed PK parameters (e.g., C_{max}, AUC_{24hrs}) and tumor response will also be performed.

For phase IB part of the study, anti-tumor efficacy is considered a secondary objective. All subjects who have received a minimum of 1 cycle of study treatment, had baseline assessments and at least one on-study tumor assessment will be considered evaluable for response.

- For an individual subject, the best overall response to treatment is the best response recorded from the start of treatment until disease progression/recurrence (taking as a reference for progressive disease the smallest measurements recorded since the treatment started) or the withdrawal from the study. Stable disease will be considered the best response when observed for at least six months from baseline.
- ORR (objective tumor response rate) is defined as the proportion of subjects that experience CR and PR based on RECIST 1.1 and/or RANO-BM criteria.
- CBR (clinical benefit rate) is defined as proportion of subjects with CR, PR or SD, based on RECIST 1.1 and/or RANO-BM criteria.

- DOR (duration of response) is defined as the time from enrollment into clinical trial until objective tumor progression or death whichever occurs first.
- ORR will be reported as absolute number of subjects out of total number of subjects in the group. Descriptive summaries for ORR and DOR will be provided.
- Overall CBR for all subjects enrolled in phase IB part of the study will be calculated and reported as percentage, 95% exact CI will be provided.

11.2 Analytic plan for phase II part of the study

This is an open-label single arm study to determine efficacy, further delineate safety profile, and to identify potential biomarkers of response to a combination treatment with tucatinib, palbociclib and letrozole in HR+/HER2+ subjects.

11.2.1 Analytic plan for primary endpoint

The primary efficacy endpoint for the phase II part of the study is PFS defined as the time from allocation to the first documented disease progression according to RECIST 1.1 (for subjects with CNS disease RECIST 1.1 and/or RANO-BM) or death due to any cause, whichever occurs first. If a subject does not have a documented date of progression or death, PFS will be censored at the date of the last adequate assessment. For PFS, Kaplan-Meier quartile estimates along with the 95% CI will be provided.

Subjects from phase Ib and phase II parts of the study will be included into PFS analysis. PFS analysis will be performed both in the intent-to-treat (ITT) population (all subjects enrolled in the study regardless of treatment received), as well as in treated population of subjects (all subjects who have received a minimum of 1 cycle of study treatment, had baseline assessments and at least one on-study tumor assessment).

The study is designed to detect a 50% improvement in PFS on triple combination targeted therapy comparing to historical control. It is anticipated that 20 evaluable subjects in the phase Ib part and 20 subjects in the phase II part will be enrolled into this study. TH3resa study (TDM-1 versus physician's choice therapy) enrolled similar patient population [33, 34] and represent an appropriate study for comparison. TH3resa showed median PFS of 6.2 months in TDM-1 treatment arm. Using the survival analysis and assuming that the survival time is exponentially distributed, accrual time is 12 months, follow up time is 18 months, and drop out / loss of follow up rate is 5%, the sample size of 40 patients total (20 patients enrolled in phase Ib part, and 20 patients enrolled in phase II part) will allow us to achieve a statistical power of 82% with a one-sided type I error rate of 0.1 to detect 50% improvement in median PFS (from 6.2 month in the historical control [33, 34] to 9.3 months in the current study).

11.2.2 Analytic plan for secondary endpoints

Secondary end-points for the phase II part of the study include additional safety assessments (incidence, nature and severity of all AEs that occur on or after C1D1 of therapy), PKs, and evaluation of additional efficacy parameters (ORR, CBR and DOR).

Safety will be evaluated through summaries of AEs; AEs will be classified by system organ class (SOC) and preferred term using the Medical Dictionary for Regulatory Activities (MedDRA version 18.0); AE severities will be classified using the NCI CTCAE criteria version 4.03. In the event of multiple occurrences of the same AE with the same preferred term in one subject, the AE will be counted once as the occurrence.

Collected AE data will be summarized in the following categories:

- All AEs (regardless of grade)
- All grade 3/4/5 AEs
- All drug-related AEs (regardless of grade)
- All AEs leading to study drug or study discontinuations
- All AEs of special interest (regardless of grade) – LFT abnormalities, changes in cardiac ejection fraction, incidence of neutropenia and infections, and cerebral edema.

Additional data on extend of exposure, tolerability and compliance will be collected:

- Extend of exposure will be assessed by analyzing study drug administration data, any dose modifications or dose holdings will be flagged. For each of the regimen components (tucatinib, palbociclib and letrozole), duration of exposure and number of treated cycles will be summarized.
- For each of the regimen components (tucatinib, palbociclib, letrozole), tolerability will be assessed by tabulating the frequency of study drug discontinuations.
- For each of the regimen components (tucatinib, palbociclib, letrozole), treatment compliance (percent of actual to planned dosing) will be summarized.
- Additionally, subjects who discontinue study drug prematurely or withdraw from the study will be summarized and listed, with the reason for early termination and/or withdrawal. Major protocol violations will be summarized. All non-protocol specified anti-cancer therapies will be listed and summarized.

Additional PKs will be obtained for 10 patients enrolled in phase II part of the study. Plasma concentration/time data of the drugs obtained at the designated times will be summarized descriptively and presented graphically by dose and day of assessment. The concentration/time data will be analyzed using non-compartmental methods to obtain PK parameters in individual subjects. These PK parameters include the maximum plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}), area under the plasma concentration versus time curve to 24 hours (AUC_{24hr}) and area under the plasma concentration versus time curve to the time of the last measurable concentration (AUC_{last}). If data permits, area under the plasma concentration versus time curve to infinity (AUC_{inf}), terminal elimination half-life ($t_{1/2}$), oral plasma clearance (CL/F) and apparent volume of distribution (Vd/F) will be also estimated. Individual values and descriptive statistics of these PK parameters will be provided by dose and day of assessment in tabular form. Exploratory analyses investigating the relationship between computed PK parameters (e.g., C_{max}, AUC_{24hrs}) and tumor response may also be performed.

Efficacy evaluation include assessment of ORR, CBR and DOR. Subjects from both phase I and phase II part of the study will be included into the analysis, and analysis will be performed in both intend to treat and treated population of subjects as it was previously described in section 11.1.2.

11.3 Early stopping rules

There are no early stopping rules for futility, given the known activity of the standard and experimental drugs in this trial. Early stopping of the protocol for safety reasons could occur if the pre-planned safety analyses from the Phase IB portion of the study are not satisfactory or at any time during phase II if unexpected significant toxicities are identified which will be determined, if this unlikely event should occur, by input from the Lead PI, medical monitor, Pfizer Inc, Seattle Genetics, Inc. and/or the University of Colorado Cancer Center DSMC.

11.4 Sample size justification

The study is designed to detect a 50% improvement in PFS on triple combination targeted therapy comparing to historical control. It is anticipated that 20 evaluable subjects in the phase Ib part and 20 subjects in the phase II part will be enrolled into this study. TH3resa study (TDM-1 versus physician's choice therapy) enrolled similar patient population [33, 34] and represent an appropriate study for comparison. TH3resa showed median PFS of 6.2 months in TDM-1 treatment arm. Using the survival analysis and assuming that the survival time is exponentially distributed, accrual time is 12 months, follow up time is 18 months, and drop out / loss of follow up rate is 5%, the sample size of 40 patients total (20 patients enrolled in phase Ib part, and 20 patients enrolled in phase II part) will allow us to achieve a statistical power of 82% with a one-sided type I error rate of 0.1 to detect 50% improvement in median PFS (from 6.2 month in the historical control [33, 34] to 9.3 months in the current study).

12 DATA SAFETY MONITORING

The Lead PI and/or Criterium will be responsible for clinical trial monitoring, overseeing the safety of the trial, including any specimens collected, executing the Data and Safety Monitoring (DSM) Plan, and overseeing the compliance with all reporting requirements to local and federal authorities. This will be accomplished through an additional oversight from the DSMC at the University of Colorado Cancer Center. The DSMC is responsible for ensuring data quality and study participant safety for all clinical studies at the University of Colorado Cancer Center, which is the coordinating institution of this trial. A summary of the DSMC's activities is as follows:

- Conduct of internal audits
- Ongoing review of all SAEs, unanticipated problems (UAPs) and reportable AEs
- Has the authority to close and/or suspend trials for safety or trial conduct issues
- May submit recommendations for corrective actions to the CU Cancer Center's Executive Committee

Per the University of Colorado Cancer Center Institutional DSM Plan, SAEs, UAPs and reportable AEs are reported to the DSMC, COMIRB and the study sponsors per protocol. All SAEs, UAPs and reportable AEs are to be reported to the DSMC within 5 business days of the Lead PI receiving notification of the occurrence.

The study Lead PI is responsible for organizing and conducting monthly teleconferences with all participating sites to discuss patients' enrollment and safety of the trial. Each subject's treatment outcomes, data regarding number of subjects, significant toxicities, dose modifications, and treatment responses will be discussed and documented in the meeting's minutes.

The Lead PI will provide a DSM report to the University of Colorado Cancer Center DSMC every six months including data from all participating sites. Criterium will assist in generating DSM report data from the study database. The DSM report will include a protocol summary; current enrollment numbers, summary of toxicity data to include specific SAEs, UAPs and AEs; any dose modifications; all protocol deviations and protocol amendments. The lead study PI is also responsible for overseeing the efficacy of the trial, therefore, the DSM report will include, if applicable, the results of any efficacy data analysis conducted. Results and recommendations from the review of this six month report by the University of Colorado Cancer Center DSMC will then be provided to the Lead PI in a DSMC review letter. The Lead PI is then responsible for ensuring this letter is provided to each participating site. Each participating site will be responsible for submitting the results and recommendations from the DSMC's six month review to their IRB of record at the time of IRB continuing review per IRB policy.

13 QUALITY CONTROL AND QUALITY ASSURANCE

13.1 Clinical site monitoring

Remote site monitoring will be conducted to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, verifiable, within protocol requirements, and congruent across the data, and that the conduct of the trial is in compliance with the currently approved protocol/ amendment(s), with GCP, and with applicable regulatory requirement(s). Site monitoring will be performed remotely by Criterium pursuant to the Remote Monitoring Plan (RMP), incorporated herein by reference. The RMP describes in detail who will conduct the remote monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of the monitoring reports/summary Note to Files. Independent audits may be conducted by the University of Colorado Cancer Center DSMC to ensure safety of the clinical trial participants and consistency of clinical trial procedures and data collection across all participating sites. In addition, audits may be conducted at any time by appropriate regulatory authorities and/or the IRBs.

13.2 Quality control (QC) procedures

Quality Control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run as the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/ resolution.

Following written SOPs, the study monitor will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial-related sites, source data/ documents, and reports for the purpose of monitoring and, if needed, auditing by the DSMC audit team, and inspection by local and regulatory authorities.

14 DATA HANDLING AND RECORD KEEPING

14.1 Case Report Forms/Electronic data record

The term case report form (CRF) should refer to either a paper form or an electronic data record or both. A CRF is required and should be completed for each included subject. The completed original CRFs are property of the University of Colorado Denver, Lead PI and appropriate regulatory authorities. It is the investigator's responsibility to ensure completion and to review and approve all CRFs. CRFs must be signed by the investigator or by an authorized staff member. These signatures serve to attest that the information contained on the CRFs is true. At all times, the investigator has final personal responsibility for the accuracy and authenticity of all clinical and laboratory data entered on the CRFs. Subject source documents are the physician's patient records maintained at the trial site.

14.2 Record retention

As described in the ICH GCP Guidelines, “essential documents”, including CRFs or eCRFs, source documents, Informed Consent Forms (ICFs), laboratory test results and the investigational product inventory records should be retained by the investigator until at least two years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least two years have elapsed since the formal discontinuation of clinical development of the investigational product.

These documents should be retained for a longer period however if required by the applicable regulatory requirements or by an agreement with Pfizer and/or Seattle Genetics, Inc..

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial be retained by the Investigator until notified by Pfizer and Seattle Genetics, Inc. in writing that retention is no longer necessary. These records must be made available at reasonable times for inspection and duplication, if required, by a properly authorized representative of the US FDA, European Competent Authorities, and Health Canada (HC) in accordance with regulatory requirements.

To protect the safety of study subjects and to ensure accurate, complete, and reliable data, the investigator will keep records of laboratory tests, clinical notes, and subject medical records in the subject files as original source documents. If requested, the Investigator will provide Pfizer and Seattle Genetics, Inc., applicable regulatory agencies, and applicable IRB/IEC with direct access to the original source documents. Records containing subject medical information must be handled by the Investigator in accordance with the required privacy policies and consistent with the terms of the subject authorization contained in the informed consent document for the study. Care should be taken to ensure that such records are not shared with any person or for any purpose not contemplated by the authorization. Furthermore, eCRFs and other documents to be transferred to Pfizer, Seattle Genetics, Inc. or their designee should be completed in strict accordance with the instructions regarding the coding of subject identities. No study document should be destroyed without prior written agreement between Pfizer, Seattle Genetics, Inc. and the investigator. Should the investigator wish to assign the study records to another party or move them to another location, written approval must be obtained from Pfizer and Seattle Genetics, Inc.. The Investigator must keep these documents on file according to local regulations after completion or discontinuation of the study. After that period of time the documents may be destroyed, subject to local regulations.

Should the Investigator wish to assign the study records to another party or move them to another location, Pfizer and Seattle Genetics, Inc. must be notified in advance. If the Investigator cannot guarantee this archiving requirement at the investigational site for any or all of the documents, special arrangements must be made between the investigator and Pfizer and Seattle Genetics, Inc. to store these in a sealed container(s) outside of the site so that they can be returned sealed to the investigator in case of a regulatory audit. Where source documents are required for the continued care of the subject, appropriate copies should be made before storing outside of the site.

15 ETHICS

15.1 Institutional Review Board (IRB)

The trial protocol, protocol amendments, informed consent form(s), and other relevant documents (i.e., recruitment materials, and all subject materials) will be submitted to each site's designated IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any subject is enrolled at the site. Any amendment to the protocol will require review and approval by the site's IRB before the changes are implemented to the study. All changes to the consent form will require the site's IRB approval and determination whether previously consented subjects need to be re-consented to an amended consent form. All correspondence with the IRB should be retained in the site's Investigator File. The only circumstance in which an amendment may be initiated prior to IRB approval is where the change is necessary to eliminate apparent immediate hazards to the subjects. In that event, the site

investigator must notify the Lead PI and their IRB in writing within 5 working days after the implementation.

15.2 Ethical conduct of the trial

The trial will be performed in accordance with the protocol, and applicable federal, state and local regulatory requirements and laws.

15.3 Subject information and consent

The informed consent process will be initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. All parties will ensure protection of subject personal data and will not include subject names on any forms, reports, publications, or in any other disclosures. The informed consent form (ICF) will be approved by the site's IRB and must be in compliance with regulatory and legal requirements. The investigator must ensure that each trial subject, or his/her legally authorized representative is fully informed about the nature and objectives of the trial. The investigator will discuss the risks and possible benefits of study participation in detail with subjects and their families.

The investigator, or a person designated by the investigator, will obtain written informed consent from each subject or, if permitted by state laws, the subject's legally authorized representative, before any trial-specific activity is performed. The informed consent form used in this trial and any changes made during the course of the trial must be prospectively approved by the IRB before use. The investigator will retain the original of each subject's signed consent form.

16 DEFINITION OF END OF TRIAL

End of trial is defined as the time at which:

- Enrollment is completed according to protocol planned sample size, and assessment and requirements are completed as per protocol
- The stated objectives of the trial are achieved
- Premature termination of this clinical trial may occur because of a regulatory authority decision, change in opinion of the IRB/IEC, drug safety problems, or at the discretion of Lead PI, Criterium, Pfizer, Inc. or Seattle Genetics, Inc.

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18 APPENDIX

18.1 Appendix A: List of abbreviations

Abbreviation	Term
AE	Adverse Event
AI	Aromatase Inhibitor
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
ASCO	American Society of Clinical Oncology
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BID	Twice a Day
CABG	Coronary Artery Bypass Graft
CAP	College of American Pathologists
CBR	Clinical Benefit Rate
C1D1	Cycle 1 Day 1
CDK	Cyclin Dependent Kinase
CHF	Congestive Heart Failure
C_{max}	Maximum plasma Concentration
CMP	Clinical Monitoring Plan
CNS	Central Nervous System
COMIRB	Colorado Multiple Institutional Review Board
CR	Complete Response
CRF	Case Report Form
CT	Computer Tomography
ctDNA	Circulating Tumor DNA
DL	Dose Level
DOR	Duration of Response
DSM	Data Safety Monitoring
DSMC	Data Safety Monitoring Committee
ECHO	Echocardiogram
ECOG	Eastern Cooperative Oncology Group
EGFR	Epidermal Growth Factor Receptor
EKG (ECG)	Electrocardiogram
ER	Estrogen receptor
ERα	Estrogen receptor alpha
ESR1	Estrogen receptor gene
FDA	Food and Drug Administration
FFPE	Formalin Fixed Paraffin Embedded
IC_{50}	Half Maximal Inhibitory Concentration
ICF	Informed Consent Form
IHC	Immunohistochemistry
IHC GCP	International Conference on Harmonization Good Clinical Practice

INR	International Normalized Ratio
IRB	Institutional Review Board
ISH	In Situ Hybridization
HER	Human Epidermal Growth Factor Receptor
HR	Hormone Receptor
hERG (assay)	Either-a-go-go Related Gene Assay
LFTs	Liver Function Tests
LVEF	Left Ventricular Ejection Fraction
MeDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic Resonance Imaging
MTD	Maximum Tolerated Dose
MUGA (scan)	Multiple Gated Acquisition Scan
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
ORR	Overall Response Rate
OS	Overall Survival
pCR	Pathologic Complete Response
PD	Progressive disease
PET	Positron Emission Tomography
PFS	Progression Free Survival
PI	Principle Investigator
PIC	Powder in Capsule
PK	Pharmacokinetics
PO	Orally
PR	Progesterone Receptor
PR	Partial Response
PS	Performance Status
PT	Prothrombin Time
PTCA	Percutaneous Transluminal Coronary Angioplasty
PTT	Partial Thromboplastin Time
QTc	Corrected QT Interval
Rb	Retinoblastoma (protein)
RECIST	Response Evaluation Criteria in Solid Tumors
RP2D	Recommended Phase 2 Dose
SAE	Serious Adverse Event
SD	Stable Disease
SNV	Single Nucleotide Variant
SOC	System Organ Class
TEAE	Treatment Emergent Adverse Event
TTP	Time to Progression
UAP	Unanticipated Problem
UGT1A1	UDP-glucuronosyltransferase 1A1
ULN	Upper Limit of Normal

UTI	Urinary Tract Infection
WBC	White Blood Cell Count

18.2 Appendix B: Performance status

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active 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.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

18.3 Appendix C: RECIST 1.1 criteria, RANO-BM criteria and bi-compartmental assessment of response

18.3.1 RECIST criteria for solid tumors version 1.1

Selected sections from RECIST 1.1 criteria for solid tumors are provided below. For detailed guidelines, see complete RECIST 1.1 criteria [26].

Measurability of Tumor Lesions at Baseline

Definitions

At baseline, tumor lesions will be categorized as follows: measurable (lesions that can be accurately measured in at least one dimension [longest diameter to be recorded] as 20 mm with Xray, 10 mm by caliber on physical exam, or 10 mm with CT scan) or non-measurable (all other lesions, including small lesions and truly non-measurable lesions). To be considered pathologically enlarged and measurable, a lymph node must be at least 15 mm in short axis when assessed by CT scan. All measurements should be recorded in metric notation by use of 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 (approximately 30 days) before the beginning of treatment. Lesions considered to be truly non-measurable include the following: bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis / pulmonis, and abdominal masses identified by physical exam that are not confirmed by reproducible imaging techniques. Note: Tumor lesions that are situated in a previously irradiated area are usually considered non-measurable, unless there has been a demonstrated progression in the area.

Specifications by Methods of Measurements

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 when both methods have been used to assess the anti-tumor effect of a treatment.

- Clinical Examination – clinically detected lesions will be considered measurable only when they are superficial (e.g., skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is required.
- Chest X-ray – lesions on chest X-rays are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung.
- CT and MRI – CT and MRI are the best currently available and most reproducible methods for measuring target lesions selected for response assessment. RECIST 1.1 guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness.
- 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.
- Endoscopy and Laparoscopy – the utilization of these techniques for objective tumor evaluation is not advised. However, such techniques can be useful in confirming complete histopathologic response when biopsy specimens are obtained.
- Tumor Markers – tumor markers alone cannot be used to assess response. However, if markers are initially above the upper normal limit, they must return to normal levels for a subject to be considered in complete clinical response when all tumor lesions have disappeared.

Tumor Response Evaluation

Baseline Evaluation

Assessment of Overall Tumor Burden and Measurable Disease:

To assess objective response, it is necessary to estimate the overall tumor burden at baseline to which subsequent measurements will be compared. Measurable disease is defined by the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

Baseline Documentation of "Target" and "Non-target" Lesions:

All measurable lesions up to a maximum of 5 lesions per organ and 10 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 (those with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameters for all target lesions will be calculated and reported as the baseline sum of the longest diameters. To calculate the sum of the longest diameters of all target lesions, add up longest diameters of non-nodal target lesions (axial plane), and add short axis diameters of target lymph nodes. The baseline sum of the longest diameters will be used as the reference by which to characterize the objective tumor response. All other lesions (or sites of disease) should be identified as non-target lesions and should be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

Response Criteria

Evaluation of Target Lesions:

- CR—the disappearance of all target lesions; any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
- PR—at least a 30% decrease in the sum of the longest diameters of target lesions, taking as reference the baseline sum of the longest diameters;
- PD—at least a 20% increase in the sum of the longest diameters of target lesions, taking as reference the smallest sum of the longest diameters recorded since the treatment started or the appearance of one or more new lesions. The sum of the longest diameters must also demonstrate an absolute increase of at least 5mm (ex.: two lesions increasing from 2 mm to 3 mm do not qualify). The appearance of one or more new lesions is PD.
- SD—neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as a reference the smallest sum of the longest diameters since the treatment started.

Evaluation of Non-target Lesions:

- CR—the 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)
- Non-CR / Non-PD – persistence of one or more non-target lesion(s) and/or the maintenance of tumor marker level above the normal limits
- PD—the appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. Note: disease progression based on progression of "non-target" lesions only is exceptional. In this setting, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest increase in the size of one or more non-target lesions is usually not sufficient to quality for unequivocal progression status. Appearance of the new lesions will signify PD.

Evaluation of Best Overall Response:

The best overall response is the best response recorded from the start of treatment until disease progression/recurrence (taking as a reference for PD and SD the smallest measurements recorded since the treatment started). The table below provides overall responses for all possible

combinations of tumor responses in target and non-target lesions with or without the appearance of new lesions.

Overall responses for all possible combinations of tumor responses in target and non-target lesions with or without the appearance of new lesions

Target Lesions	Non-target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR / Non-PD	No	PR
PR	Non-CR / Non-PD	No	PR
SD	Non-CR / Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

CR – complete response; PR – partial response; SD – stable disease; PD – progressive disease

18.3.2 RANO-BM criteria

Selected sections from RANO-BM criteria are provided below. For detailed instructions, see published guidelines [27].

CNS vs. non-CNS assessments: Assignment of CNS response and progression is independent of non-CNS response and progression. Non-CNS response and progression will be assessed by RECIST 1.1. Assessment of CNS response and progression will be per the RANO-BM criteria. At each assessment point where a CNS assessment occurs, the MRI brain should be coincident with a corresponding non-CNS assessment.

Measurable lesions: Up to five measureable CNS lesions may be identified as target lesions. A sum of longest diameters for all target lesions will be recorded at baseline and at each subsequent measurement. Definition of measurable disease:

- Contrast enhancing lesion that can be measured accurately in at least one dimension with a minimum size of 10 mm, and visible in 2 or more axial slices that are ≤5 mm apart with 0 mm skip
- The diameter perpendicular to the longest diameter must be ≥5 mm
- Cysts and surgical cavities are not measurable unless there is a nodular component measuring ≥10 mm in the longest diameter and ≥5 mm in the perpendicular plane
- Lesions previously treated with local therapy (SRS, WBRT, or surgery) may only be considered measurable if progression has occurred since the time of local treatment. If lesions are present which have not been treated previously with local therapy, these are preferred target lesions
- All other lesions should be considered non-measurable and listed as non-target lesions, including dural metastases, bony skull metastases, and cystic-only lesions. At each assessment, these should be classified as present (Non-CR / Non-PD), absent (CR), or unequivocal progression.

Lesions < 5 mm: For measurable lesions responding to therapy which are sufficiently small (but still present) to be assigned an exact measure, a default value of 5 mm should be recorded. If a lesion disappears, the value should be recorded as 0 mm.

Coalescence of lesions: Lesions might coalesce during treatment. As lesions coalesce, a plane between them may be maintained that would aid in obtaining maximum longest diameter of each individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximum longest diameter for the coalesced lesion.

New lesions: The finding of a new CNS lesion should be unequivocal and not due to technical or slice variation. A new lesion is one that was not present on prior scans. If the MRI is obtained with slice thickness of 1.5 mm or less, the new lesion should also be visible in axial, coronal, and sagittal reconstructions of 1.5 mm or thinner projections. If a new lesion is equivocal, for example because of its small size (i.e., ≤5 mm), continued therapy can be considered, and a follow-up assessment will clarify if it really is new disease. If repeated scans confirm a new lesion, progression should be declared using the date of the initial scan showing the new lesion.

Treatment effect from radiation or surgery and pseudo-progression: this applies to subjects who have been treated with radiation or surgery, for whom there has been radiographic evidence of enlargement of target or non-target lesions, which do not necessarily represent tumor progression. If radiographic evidence of progression exists, but clinical evidence indicates that the radiological changes are due to treatment effect (and not to progression of cancer), additional evidence is needed to distinguish between true progression and treatment effect, in which a single MRI alone is insufficient. Subjects can be continued on protocol therapy pending further investigation as follows:

- The scan can be repeated at the next protocol-scheduled assessment or sooner, and generally within about 6 weeks. An investigator can choose a shorter time interval if progressive symptoms or other clinical concerns arise.
- Continued tumor growth on follow up MRI is consistent with PD, in which case the subject should go off study. The date of progression will be the date of the initial scan where progression was suspected.
- Stabilization and shrinkage of a lesion can be consistent with treatment effect, in which case the subject can stay on study.
- For subjects with equivocal results even on the next restaging scan, the scan can be repeated again at a subsequent protocol-scheduled assessment or sooner. Equivocal scans must all initially be recorded as "not evaluable".

Corticosteroid use: In the absence of clinical deterioration related to the tumor, an increase in corticosteroid dose alone should not be used as a sole determinant of progression. Subjects with stable imaging results and whose corticosteroid dose has increased for reasons other than clinical deterioration related to the tumor do not qualify as having progression. These subjects should be observed closely, and if their corticosteroid dose can be reduced back to baseline, they may be considered evaluable for response, but if further clinical deterioration related to the tumor becomes apparent, they will be considered as having clinical progression. The definition of clinical deterioration is left to the discretion of the treating physician. For subjects who initiate or increase the dose of corticosteroids but who are not considered to have clinical or radiographic progression, scans taken during the use of corticosteroids in this scenario are not evaluable for response at that time point.

Response assessment of target and non-target lesions

Confirmatory scans are required at a minimum of 4 weeks after initial scan.

Target lesions

CR – disappearance of all CNS target lesions sustained for at least 4 weeks; with no new lesions, no use of corticosteroids, and subject is stable or improved clinically.

PR – at least a 30% decrease in the sum of the longest diameters of CNS target lesions, taking as a reference the baseline sum of the longest diameters sustained for at least 4 weeks; no new lesions; stable to decreased corticosteroid dose, stable or improved clinical condition.

PD – at least a 20% increase in the sum of the longest diameters of CNS target lesions, taking as a 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%, at least one lesion must increase by an absolute value of 5 mm or more to be considered progression.

SD – neither sufficient shrinkage to qualify for PR, nor sufficient increase to qualify for PD, taking as reference the smallest sum of the longest diameters while on study.

Non-target lesions

Non-target lesions should be assessed qualitatively at each of the time points per protocol.

CR – requires all of the following: disappearance of all enhancing CNS non-target lesions, no new CNS lesions.

Non-CR / Non-PD – persistence of one or more non-target CNS lesions.

PD – any of the following: unequivocal progression of existing enhancing non-target CNS lesions, new lesion(s), or unequivocal progression of existing tumor-related non-enhancing (T2/FLAIR) CNS lesions.

Summary of the Response Criteria for CNS Metastases by RANO-BM

	CR	PR	SD	PD
Target lesions	None	≥ 30% decrease in sum of the longest diameters relative to baseline	< 30% decrease relative to baseline but < 20% increase in sum of the longest diameter relative to nadir	≥ 20% increase in sum of the longest diameters relative to nadir; at least one lesion must increase by an absolute value of 5mm or more
Non-target lesions	None	Non-CR / Non-PD	Non-CR / Non-PD	Unequivocal PD
New lesions	None	None	None	Present
Corticosteroids	None	Stable or decreased	Stable or decreased	Not applicable*
Clinical status	Stable or improved	Stable or improved	Stable or improved	Worse
Requirement for response	All	All	All	Any

* Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration.

18.3.3 Bi-compartmental (CNS and non-CNS) response assessment

CNS (RANO-BM)	Non-CNS (RECIST 1.1)	Response
CR, PR or SD	CR, PR or SD	Record as CNS and non-CNS CR, PR or SD
CR, PR or SD	PD	Record as CNS CR, PR or SD; record as non-CNS PD
PD	SR, PR or SD	Record as CNS PD; record as non-CNS SR, PR or SD
PD	PD	Record as both CNS and non-CNS PD

18.3.4 Bi-compartmental progression-free survival

CNS (RANO-BM)	Non-CNS (RECIST 1.1)	Bi-compartmental PFS	Note
CR, PR or SD	PD	Record as PFS event	Record as non-CNS PD
PD	CR, PR or SD	Record as PFS event	Record as CNS PD
PD	PD	Record as PFS event	Record as both CNS and non-CNS PD

In this trial, if a subject is found to have progressive disease due to CNS progression per RANO-BM, but does not have progressive disease outside the CNS per RECIST 1.1, the subject may undergo local therapy (radiotherapy or surgery) to the progressive CNS lesion(s), and continue on study after discussion with and approval from the medical monitor. The subject may continue on study until a second progressive disease event occurs (either CNS or non-CNS).

18.4 Appendix D: List of prohibited medications and medication that require caution

It is recommended that concomitant medication check will be performed by a clinical pharmacist prior to subject enrollment in the trial to verify that subject is not taking any concomitant medication that belongs to the groups of prohibited medications listed in the Appendix D.

The following medications are prohibited during the entire study period and within 5 elimination half-lives prior to first dose of study treatment:

- strong CYP3A4 inducers or inhibitors (potential interaction with tucatinib and palbociclib)
- strong CYP2C8 inducers or inhibitors (potential interaction with tucatinib)
- moderate CYP2C8 inhibitor trimethoprim (prohibited due to prior adverse events on a clinical trial when used in combination with tucatinib)

CYP3A STRONG INDUCERS (PROHIBITED)

Carbamazepine (Tegretol®)
Phenobarbital
Phenytoin (Dilantin®)
Rifampin
St. John's Wort

CYP3A STRONG INHIBITORS (PROHIBITED)
Clarithromycin
Conivaptan
Diltiazem
Fluvoxamine
Grapefruit Juice (in large amounts, > 1 liter a day, and in high concentrations)
Itraconazole
Ketoconazole
Nefazodone
Posaconazole
Quinupristin
Telithromycin
Troleandomycin
Voriconazole
Verapamil
Cranberries/cranberry juice

CYP2C8 STRONG INDUCERS (PROHIBITED)
Rifampin

CYP2C8 STRONG INHIBITORS (PROHIBITED)
Gemfibrozil
Montelukast
Quercetin
Rosiglitazone
Clopidogrel

CYP2C8 MODERATE INHIBITOR (PROHIBITED)
Trimethoprim

The following medications should be used with caution during the study period:

- Moderate inhibitors or inducers of CYP3A4 (potential interaction with tucatinib and palbociclib)
- Moderate inhibitors of CYP2C8 other than trimethoprim (potential interaction with tucatinib)
- Sensitive substrates of CYP3A4 (potential interaction with tucatinib). Avoid concomitant use of sensitive CYP3A4 substrates with tucatinib when possible, consider using an alternate medication which is not a sensitive CYP3A4 substrate. If the use of additional sensitive CYP3A substrates is unavoidable, consider dose reduction of CYP3A4 substrates with narrow therapeutic indices and/or increased monitoring for potential adverse reactions as described in the medication's prescribing information.
- Inhibitors or substrates of P-gp (potential interaction with tucatinib)
- Substrates of BCRP (potential interaction with tucatinib)
- Sensitive substrates of UGT1A1 (potential interaction with tucatinib)

CYP3A MODERATE INDUCERS
Bosentan
Modafinil (Provigil®)
Nafcillin

CYP3A MODERATE INHIBITORS
Aprepitant (Emend®)
Cimetidine
Ciprofloxacin (Cipro®)
Clotrimazole
Cyclosporine
Dronedarone (Multaq®)
Erythromycin
Fluconazole (Diflucan®)
Tofisopam

CYP2C8 MODERATE INHIBITORS
Deferasirox
Teriflunomide

SENSITIVE CYP3A SUBSTRATES
Alfetanil
Apixaban (Eliquis)
Avanafil
Budesonide
Buspirone
Conivaptan
Darifenacin
Dronedarone
Ebastine
Eletriptan
Eplerenone
Felodipine
Lurasidone
Lomitapide
Lovastatin
Lurasidone
Midazolam
Naloxegol
Nisoldipine
Quetiapine
Rivaroxaban (Xarelto)
Sildenafil
Simvastatin
Ticagrelor
Tolvaptan

Triazolam
Vardenafil

Many anti-HIV and anti-Hep C medications are strong or moderate inhibitors or inducers or sensitive substrates of CYP3A, they are not included in this list because patients with chronic hepatitis and HIV are excluded this study.

INHIBITORS OF P-GP
Quinidine
Reserpine

SUBSTRATES OF P-GP
Dabigatran (Pradaxa)
Digoxin
Fexofenadine
Loperamide
Quinidine

SUBSTRATES OF BRCP
Dantrolene
Rosuvastatin
Prazozin
Sulfasalazine

SENSITIVE SUBSTRATES OF UGT1A1
Irinotecan is a sensitive substrate of UGT1A1; however, it will not be used in this study (prohibited as one of anti-cancer agents)

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COMIRB No: **16-1661**

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Study Title: **PHASE IB/II OPEN-LABEL SINGLE ARM STUDY TO EVALUATE SAFETY AND EFFICACY OF TUCATINIB IN COMBINATION WITH LETROZOLE AND PALBOCICLIB IN SUBJECTS WITH HORMONE RECEPTOR POSITIVE AND HER2-POSITIVE METASTATIC BREAST CANCER**

You are being asked to be in a research study. This form provides you with information about the study. A member of the research team will describe this study to you and answer all of your questions. Please read the information below and ask questions about anything you do not understand before deciding whether or not to take part.

Why is this study being done?

This study plans to learn more about the combination therapy of tucatinib with palbociclib and letrozole and how these drugs work together to treat your type of cancer. Letrozole has been approved as standard treatment for metastatic breast cancer by the U.S. Food and Drug Administration (FDA). Palbociclib has been approved by the FDA for treatment of hormone receptor positive (HR+) metastatic breast cancer, but has not yet been approved for patients with HR+ and HER2+ metastatic breast cancer. Tucatinib has not been approved for combination treatment of Palbociclib and letrozole in metastatic breast cancer patients. However, Tucatinib has been approved for combination treatment with trastuzumab and capecitabine for patients with advanced unresectable or metastatic HER2+ breast cancer. This is considered an “Investigational” study because this combination of drugs has not been approved by the FDA. The purpose of this study is to evaluate the safety and how well the study drugs are tolerated when given in combination.

You are being asked to be in this research study because you have been diagnosed with HR+/HER2+ metastatic breast cancer.

Throughout the rest of this form, when these drugs are referenced by themselves they will be called tucatinib, palbociclib and letrozole, but when referenced together in combination use will be called the **“study drug(s)”.**

Other people in this study

This study has two parts. The first part is a dose escalation, also called Phase Ib. The second part is an expansion, also called Phase II. These parts will be described in the next section of this consent form.

Up to 25 people from around the country will participate in the Phase II part of this study.

Up to 50 people from around the country will participate in both parts of the study.

What happens if I join this study?

If you join the study, you will be asked to sign this consent form. You will be given a copy to keep and the original form will be kept at the clinic. You can withdraw from the study at any time and without giving a reason. This will not affect the standard medical care you receive.

The two parts to this study are:

Part I - Phase Ib
Dose de-escalation group (cohort) – This part of the study has already taken place. The goal of this part of the study was to see how safe tucatinib is when taken in combination with palbociclib and letrozole.

Part II – Phase II
Expansion group (cohort) – In this part of the study we will use the doses of the study drugs determined from Part I. We will further study how effective this combination of study drugs may be in treating HR+/HER2+ metastatic breast cancer.

By signing this consent form, you will be taking part in Phase II of this study, which has three (3) sections:

1. Before starting the study (Screening)
2. During the Study (Treatment)
3. Completion of Study (End of Treatment and Follow-up)

There are also optional parts of this study. These optional procedures are voluntary and are not required. You can still take part in the main study if you choose not to take part in the optional study procedures. You will be given the choice later in this consent form to decide if you would like to take part in these optional procedures.

This next section is an overview of what will be expected of you, and what you can expect if you take part in this study.

Study Procedures:

Below are the study procedures and schedule of events (when each procedure will take place) for the dose expansion group (Part II) of this study. Some procedures you receive while taking part in this study are "standard of care procedures" for treatment of your disease. If you have had some of these procedures recently, they may not need to be repeated. Some procedures are required only for this research study and will be called "research" procedures. The time points when these study procedures will take place are specified in the next section called "Study Visits".

- **Informed Consent**
  This informed consent form will be discussed with you and you will be given a copy of this document. If you join the study, you will be asked to sign this consent form before you receive any study related tests or procedures for Part II.

- **Medical and Cancer History**
  Before you start the study, we will record your date of birth, race, ethnicity, and complete medical history. This medical history will look at the background and history of your cancer and any treatments you have received for your disease.

- **Physical Examination**
  A physical examination will be completed as part of your standard of care at screening and throughout the duration of the study. After you join the study, we will assess if the study drugs are is affecting your body functions including lungs, heart, abdomen, extremities, skin, head (eyes, ears, nose, hair, etc.) and your neurological function.

- **Tumor Tissue Samples**
  - Archived Tissue. If you had surgery for your cancer in the past, you must agree to allow us to contact the institution where you had your surgery and ask them to send us a portion of your tumor tissue that they have stored so we may use it for this research.
  - Tumor tissue biomarker testing during the study.
    We will ask you to allow us to take a fresh biopsy of your tumor tissue and keep it for research. There are three optional tumor biopsies in this study:

    (1) prior to the start of treatment,
    (2) after completion of the first 28 days of treatment, and
    (3) at the end of the study.

This is a voluntary part of this study, and you will be given the option later in this consent form to take part, or not take part, in this optional procedure.

- **Blood and Urine Samples**
  These tests are sometimes called safety labs so the study doctor can be sure it is safe for you to take part in this study and to be given the study drugs. These tests will include some standard of care tests and some research tests:
  - Pregnancy test (women who are able to become pregnant). A positive pregnancy test prior to being given the study drugs, will exclude you from starting or continuing to take part in the study.
  - Complete blood count (CBC)
  - Comprehensive metabolic panel (CMP)
  - Blood clotting tests (PT/INR, and PTT)
  - Biomarker blood test. This research test will look at how and if your cancer is responding to the treatment.

  Depending on when you are enrolled into the study, you may be required to do additional blood tests on Cycle 1 Day 9 and Cycle 2 Day 9. Your doctor will let you know if you will doing a pharmacokinetic test described below:
  - Pharmacokinetic (PK) blood test. This is a research related test that will allow us to see how long the study drugs remain in your body.
  - If you are required to take a PK blood test, you will be asked to have a high protein/high calorie meal (such as half and half, cream, high fat yogurt, butter, ice-cream, cheese or cream cheese) at 8 PM before taking palbociclib at 10 PM on a day prior to PK testing days.
  - Prior to PK blood test on Cycle 1 Day 9, you will not be allowed to take one of the study medications (tucatinib), but will be taking palbociclib and letrozole.
  - You will start tucatinib on Day 9 of Cycle 1 after PK blood tests are completed.

  Table 1 below provides detailed information on the timing of these tests if you are required to have the PK tests.

Table 1. Schedule of pharmacokinetic assessments

Cycle	Day	Time point
1	1-8	Participant takes only palbociclib and letrozole. Tucatinib is on hold.
1	8	Participant eats high calorie/high protein meal at 8 PM and takes palbociclib at 10 PM in the evening. Letrozole can be taken any time during the day. Record the timing of drug administration in drug diary.
1	9	~8 AM: blood draw
		~11 AM: blood draw
		~2 PM: blood draw
		~5 PM: blood draw
1	9	After completion of PKs, participant begins taking tucatinib and continues taking palbociclib and letrozole.
2	8	Participant eats high calorie/high protein meal at 8 PM and takes palbociclib at 10 PM in the evening. Letrozole can be taken any time during the day. Record the timing of drug administration in drug diary.
2	9	~8 AM: blood draw
		~11 AM: blood draw
		~2 PM: blood draw
		~5 PM: blood draw

Your doctor may order additional blood tests that he/she feels are needed to plan treatment, dose modification, or further evaluations.

- **Electrocardiogram (ECG)**
  This non-invasive procedure records the electrical activity of the heart. Electrodes are placed on the skin of the chest and connected in a specific order to a machine. Output usually appears on a long scroll of paper that displays a printed graph. This will check electrical activity of your heart.

- **Echocardiogram (ECHO) or Multigated Acquisition (MUGA) Scan**
  This is a noninvasive scan of the heart using sound waves. This test will be used to see how well your heart pumps blood.

- **Vital Signs**
  We will take your blood pressure, heart rate, respiratory rate, body temperature and weight. Height will be measured only during screening.

- **Performance Status**
  We will assess how well you are performing your daily activities.

- **Other Medications**
  Your study doctor will let you know which other medications you can and cannot take while taking part in this study. You should check with your doctor before taking any new medications. From the time you first receive the study drugs through 30 days after the last dose, we will record other medications you may be taking.

- **Review of the Adverse Events (AEs)**
  Some risks have been identified because of the disease process or through use of the study drugs. These are commonly called side effects and will be followed very closely by your doctor and the study staff. More information about these will be provided in the Risk section of this consent form.

Study Visits:

1. SCREENING

After signing this consent form you will have the following done to see if you can be in this study:

- Review your medical and cancer history
- ECHO or MUGA scan of your heart
- Physical exam
- Vital signs
- Performance status
- Blood tests:
  - Complete blood count (CBC) including types – white cells, red cells and platelets (differential) and comprehensive metabolic panel (CMP)
  - Blood clotting (PT/INR and PTT) – **research**
  - Pregnancy (women of childbearing potential)
  - Biomarker blood sample – **research**
- Review of medications you are taking
- ECG – **research**
- Imaging studies to document sites of disease and assess tumor burden. This may include diagnostic quality CT scan and bone scan to document the locations of the cancer in your body. It may also include a brain MRI, if one is necessary.
- Tumor tissue collections of archived biopsies – **research**
- Tumor tissue biopsy - **optional research procedure**

You do not have to have this procedure if you do not want to. You will be given the choice later in this consent form to decide if you want to take part in this part of the study.

If the procedures above show that you are eligible to participate in the study, you will undergo the following procedures while on treatment.

2. TREATMENT

Cycle 1 Day 1

- Review of AEs
- Review of medications you are taking
- Physical examination
- Vital signs
- Performance status
- Blood tests – CBC with differential and CMP
- Begin treatment with the study drugs once instructed at clinic visit – **research**
- You will be given a drug diary to complete – **research**

Cycle 1 Day 8

- Preparation for PK Test (*if required*) – **research**

Cycle 1 Day 9

- PK Test (*if required*) – **research**

Cycle 1 Day 15

- Review of AEs
- Review of medications you are taking
- Vital signs
- Blood tests – CBC with differential
- Review drug diary – **research**
- Continue treatment with the study drugs once instructed at clinic visit – **research**

Cycle 2 Day 1

- Review of AEs
- Review of medications you are taking
- Physical examination
- Vital signs
- Performance status
- Blood tests
  - CBC with differential and CMP
  - Biomarker blood sample – **research**
- Review drug diary from previous cycle – **research**
- Treatment with the study drugs once instructed at clinic visit – **research**
- You will be given a new drug diary to complete – **research**
- Tumor tissue biopsy - **optional research procedure**
  This procedure can be performed at any time during cycle 2. You do not have to have this procedure if you do not want to. You will be given the choice later in this consent form to decide if you want to take part in this part of the study.

Cycle 2 Day 8

- Preparation to PK Test (*if required*) – **research**

Cycle 2 Day 9

- PK Test (*if required*) – **research**

Cycle 2 Day 15

- Review of AEs
- Review of medications you are taking
- Vital signs
- Blood tests - CBC with differential
- Review drug diary – **research**
- Continue treatment with the study drugs once instructed at clinic visit – **research**

Cycle 3+ Day 1

- Review of AEs
- Review of medications you are taking
- Physical examination
- Vital signs

- Performance status
- Blood tests – CBC with differential and CMP
- Review drug compliance from previous cycle and drug diary
- Administer treatment with the study drugs once instructed at clinic visit
- You will be given a new drug diary to complete

Additional Study Procedures

While you are on this study, you will also have the following procedures done at regular time points.

- *Once every 8 weeks for the first 24 weeks (6 cycles), then once every 12 weeks:* Imaging studies to document sites of disease and assess tumor burden. This may include diagnostic quality CT scan and bone scan to document the locations of the cancer in your body. It may also include a brain MRI, if one is necessary.
- *Once every 12 weeks:* ECHO or MUGA scan

3. COMPLETION OF STUDY – (End of treatment and Follow-up)

You will need to return to clinic for a follow-up study visit if your disease gets worse, if you decide to withdraw from the study, or if you start a different anticancer therapy.

The following will be performed if not already performed in the prior week.

- Review of AEs
- Review of medications you are taking
- Review drug compliance from previous cycle and drug diary
- Vital signs
- Physical exam
- Performance status
- Blood tests
    - CBC with differential and CMP
    - Additional biomarker blood sample – **research**
- Imaging studies to document sites of disease and assess tumor burden. This may include diagnostic quality CT scan and bone scan to document the locations of the cancer in your body. It may also include a brain MRI, if one is necessary.
- Tumor tissue biopsy - **optional research procedure**

You do not have to have this procedure if you do not want to. You will be given the choice later in this consent form to decide if you want to take part in this part of the study.

You will also need to return to the clinic for an additional safety follow-up visit in about 30 days after you stop treatment with the study drugs. This visit will include:

- Review of AEs
- Review of medications you are taking
- Vital signs
- Physical exam
- Performance status
- Blood tests – CBC with differential, CMP and liver function tests

How long will I be on the study?

You may take part in this study for as long as you tolerate the study tests, procedures, and study treatments and your disease is stable or improving. You will continue to receive treatment with the study drugs until your disease progresses or you have an unacceptable drug-related side effect.

What are the possible discomforts or risks?

As with any study drug, side effects may occur when taking this study drug. While taking part in this study, and being treated with the study drugs, you will be watched carefully for any side effects. Some side effects may go away after you stop taking the study drug. Some side effects can be long lasting and may never go away or may even lead to death.

You should talk to your study doctor about any side effects or discomfort you may have. The study doctor may give you some medicine that will help with some side effects. The study doctor may also interrupt or discontinue the study drug.

You will be notified by your study doctor of any new side effects seen in other patients that occur during the time you are on the study. This may affect you wanting to continue in this research study.

You should avoid drinking grapefruit juice in large amounts (>34 oz. or 1 L) and eating cranberries or drinking cranberry juice while participating in this study as it could affect how your body metabolizes the drugs.

You may experience risks or discomforts when taking part in this study, which include some that are common such as low blood cell counts, infections, gastrointestinal (stomach and intestines) symptoms, fever, and fatigue, some that are rare, such as life-threatening infections and sepsis, and some that are unknown at this time. This is not a complete list of risks or discomforts. A comprehensive list of risks and discomforts is provided later in this consent document.

Palbociclib has been given to approximately 1,674 patients with breast cancer who received palbociclib together with hormonal treatment in Pfizer-sponsored clinical trials.

Serious and life-threatening infections have been observed in some patients treated with palbociclib.

In a rat study where palbociclib was administered for the lifespan of the rat, microglial cell (a type of cell located in the central nervous system) tumors were seen at blood concentrations higher than those used to treat humans. It is currently unknown what these findings observed only in male rats means for patients treated with palbociclib over time.

Based on animal studies and prior human studies with the study drugs, and/or other studies with similar types of drugs, side effects or discomforts you may experience while in this study include:

Risks of the Study Drugs:

Tucatinib

Common - in more than 20 out of 100 people

- Diarrhea
- Dizziness
- Fatigue
- Nausea
- Rash
- Weakness

Less Common - in 1 to 20 people out of 100 people

- Pain in the abdomen (belly pain)
- Alkaline phosphatase elevation
- Constipation
- Cough
- Creatinine increase (decreased kidney function)
- Headache
- Pain in arms or legs

- Chest pain
- Muscle pain
- Shortness of breath/difficulty breathing
- Potentially life-threatening abnormal liver function tests (hepatitis)
- Anemia (low hemoglobin), which may make you feel tired or weak
- Decreased appetite
- Low sodium, potassium, magnesium and phosphate levels, or level of other electrolytes in your body
- Bilirubin elevation (break down of red blood cells)
- Leg swelling
- Vomiting
- Infection in the nose, sinuses or throat (upper respiratory tract infection). You may have fever, pain, or a hard time breathing.
- Heavy sweating while sleeping (night sweats)
- Back pain
- Infection that could cause frequent and painful urination (urinary tract infection)

Rare - in fewer than 1 out of 100 people

- Weakness of the heart muscle (heart failure)
- EKG changes (change in your heart's electrical activity)/QT prolongation (takes heart longer than normal to recharge during beats)/irregular heartbeat/palpitations
- Dehydration

Most Important Side Effects -

- **Liver Function Problems**
  Some participants who took tucatinib had problems with their liver. Your doctor will run tests to check that your liver is working properly during your treatment with tucatinib. Your doctor may change your tucatinib dose based on the test results.

- **Diarrhea**
  Tucatinib can cause severe watery poop (diarrhea). If you have watery poop, tell your doctor **right away**. Your doctor may change your tucatinib dose if you have watery poop.

- **Kidney Function Problems**
  Some participants who got tucatinib had higher creatinine levels. This could mean that there are problems with your kidneys. If this happens,

it's usually within the first treatment cycle. Participants who have had increased levels of creatinine had these levels stay increased while they were getting treated. The levels went back down after treatment stopped.

- **Interactions with Other Medications**
  Tucatinib may change how your body reacts to some other drugs, which could stay in your body longer than usual. While you are taking part in this study, you might need to stop some medications or replace them with other medications. If you can't stop taking these medications, your doctor might need to change how much you take. Your doctor might also need to watch your health more closely while you are taking part in this study.

Palbociclib

More Common - in more than 30 out of 100 people

- Decrease in neutrophil blood cells (may increase the risk for infection)
- Decrease in white blood cell count (infection fighting cells)
- Infections, including serious bacterial or viral infections or reactivation of previous infections
- Feeling tired or fatigued

Common - in 10 to 30 out of 100 people

- Anemia (low hemoglobin), which may make you feel tired or weak
- Low platelet count, which may increase your risk of bleeding or bruising
- Inflammation or sores of the mouth or throat, which may make it difficult to talk, eat, or swallow
- Diarrhea
- Constipation
- Nausea
- Vomiting
- Joint pain
- Back pain
- Pain in the hands and feet
- Hair loss
- Rash
- Cough
- Shortness of breath
- Headache
- Decreased appetite

- Hot Flush
- Dizziness
- Inability to sleep (insomnia)
- Common Cold
- Fever

Less Common - in 5 to less than 10 people out of 100 people

- Abdominal pain
- Indigestion
- Dry mouth
- Weakness (asthenia)
- Swelling of the hands and feet
- Irritation or sores in the lining of hollow organs like mouth, throat, stomach, or bowels
- Pain
- Influenza- (flu-) like illness
- Muscle pain
- Pain in the muscles and bone including around the chest and neck
- Muscle cramps
- Increases in blood liver markers that may indicate liver damage, including potentially life threatening liver damage
- Dry skin
- Itching
- Mouth or throat pain
- Nosebleeds
- Impaired sense of taste
- Depression
- Falls
- Anxiety
- Increased blood pressure
- Heartburn (acid reflux)
- Increased creatinine levels that could indicate abnormal kidney function

Rare - in less than 5 out of 100 people

- Fever associated with a dangerously low level of a type of white blood cell counts (neutrophils)
- Blurred vision
- Dry eyes or watery eyes
- Interstitial lung disease / pneumonitis (an inflammation of the lungs which can cause cough and shortness of breath)

Letrozole

Common - in more than 20 out of 100 people

- Hot flashes or feeling flushed
- Joint pain or arthritis
- Mood swings
- Pain in the muscles
- Dryness in the vaginal area

Less Common - in 1 to 20 people out of 100 people

- Dizziness
- Fatigue
- Headache
- Difficulty sleeping
- Elevated blood pressure
- Elevated blood sugar
- Elevated cholesterol
- Nausea or vomiting
- Weight gain
- Night sweats
- Loss of bone mineral density, bone fracture
- Kidney and urinary bladder infections
- Vaginal bleeding

Rare - in less than 1 out of 100 people

- Constipation

Risks of Having Blood Taken

In this study, we will need to get about 9 tablespoons of blood from you over the course of the study for research. This is in addition to the standard of care blood tests. We will get blood by putting a needle into one of your veins and letting the blood flow into a vacuum tube. You may feel some pain when the needle goes into your vein. A day or two later, you may have a small bruise where the needle went under the skin.

Risks of biopsy

In this study, you will have an option to give up to 3 biopsies for research. There are some risks to taking a biopsy. There is a small chance that you could get an infection where the needle goes in. You may also experience redness, swelling, minor bleeding or bruising at the site where the cut was made or the needle inserted. You may experience mild to moderate pain at the site of the needle puncture. There is also a small chance that you

could have an allergic reaction to the numbing medicine. After your skin heals up, you may have a small scar where we take the samples.

Archival tumor tissue

A section will be taken from tumor tissue samples you may have had in the past. Since this has already been removed from you, there are no additional risks to you.

Risks of having an ECG

An electrocardiogram (ECG) is a test that records the electrical activity of the heart. Skin irritation is rare but could occur during an ECG from the electrodes or gel that is used.

Risks of Having an IV Inserted in Your Vein

In this study, we may need to insert a needle – connected to a plastic tube –into a vein in your arm. You will feel some pain when we first insert the tube into your vein. You may have some redness, swelling, or bruising where the tube goes under your skin. In some cases, this type of tube can cause an infection where it goes under the skin. In rare cases, it can cause a blood clot in the vein. You may have this tube inserted if you participate in the PK draws.

Risks Associated with Pregnancy

The use of the study drugs in pregnant females and nursing mothers has not been studied. The effects of the study drugs on human eggs and sperm has not been studied. The risks to a human fetus are unknown.

However, animal testing has shown embryo-fetal toxicity that could be a potential risk in humans for all the study drugs (tucatinib, palbociclib, and letrozole) in this study.

All female and male subjects of childbearing potential must use an acceptable form of birth control while on this study, as females must not become pregnant while taking the study drugs. Before you begin study treatment, your doctor will discuss acceptable forms of birth control with you.

Tucatinib, Palbociclib, or letrozole could hurt or kill a baby growing in the womb. You should not try to get pregnant or get your partner pregnant while you are on this clinical trial. An effective method of birth control should be used for up to 30 days after the end of this study.

If you are pregnant or breastfeeding, you cannot be in this study. We will remove anyone who becomes pregnant while on study from the study treatment immediately.

Additionally, tucatinib, palbociclib, or letrozole could hurt a breastfeeding baby. You must not breastfeed during the study and you should wait to breastfeed for up to 30 days after the end of treatment.

You should tell your study doctor **right away** if:

- you become pregnant while you are in this study
- you think that you are pregnant while taking part in this study
- you or your partner become pregnant within 30 days after being treated with tucatinib, palbociclib, and letrozole.

Birth Control Requirements

If you can get pregnant, you must use a safe birth control method while you are taking part in this study. Safe birth control methods fail less than 1% of the time. This means that if 100 people used this birth control for a whole year, at most 1 person would get pregnant. You must use safe birth control methods while you take part in the study and keep using them for 30 days after stopping all study treatment. If you do not use safe birth control methods while you take part in the study, we might decide that you cannot take part in the study anymore.

The safe birth control methods that you can use are:

- Non-hormonal intrauterine device (IUD) like Paragard
- tubal ligation for females (“tubes tied”)
- condoms/vaginal diaphragm
- vasectomy for males

Talk with your doctor about your birth control options. You may also choose to not be sexually active (complete abstinence) while you take part in the study.

If you are able to get pregnant, you must use a barrier method such as a male or female condom when you are taking part in the study. You must keep using a barrier method for 30 days after finishing treatment.

Fertility Risks

It is not known if patients in this trial will have problems getting pregnant after taking tucatinib, palbociclib, or letrozole.

Risk of Loss of Confidentiality

There is a risk that people outside of the research team will see your research information. We will do all that we can to protect your information, but it cannot be guaranteed.

The study may include risks that are unknown at this time.

What are the possible benefits of the study?

This study is designed for the researcher to learn more about the effects of the study drug on your disease. However, there is no guarantee that your health will improve if you join this study. Also, there could be risks to being in this study. If there are risks, these are described in the section describing the discomforts or risks.

Are there alternative treatments?

There may be other ways of treating your (HR+/HER2+) metastatic breast cancer.

These other ways include:

- You may choose to receive treatment with another experimental therapy.
- You may choose to receive treatment with another approved therapy.
  Approved therapies for patients with HR+/HER2+ metastatic breast cancer include:
  (1) anti-endocrine agents (tamoxifen, fulvestrant, anastrozole, letrozole, exemestane)
  (2) combination of anti-endocrine agents and HER2-targeted agents (trastuzumab, pertuzumab, lapatinib)
  (3) combination of chemotherapy and HER2-targeted agents
  (4) antibody-drug conjugate TDM-1
- You may choose to receive comfort/palliative care.
- You could also choose to get no treatment at all.

You should talk to your doctor about your choices. Make sure you understand all of your choices before you decide to take part in this study. You may leave this study and still have these other choices available to you.

Who is paying for this study?

This research is being conducted by Elena Shagisultanova, MD, PhD, with funding support by Pfizer, Inc. who is also providing the study drug palbociclib. Seattle Genetics, Inc. will

provide the other study drug tucatinib. You will be required to pay for your treatment with letrozole as part of your standard care.

The sponsor will only pay for procedures not considered standard of care.

Ask your study doctor to discuss any costs that will not be covered by the study. This discussion should include the costs of treating possible side effects. Otherwise, you might have unexpected expenses from being in this study

Will I be paid for being in the study?

You will not be paid to be in the study.

Will I have to pay for anything?

There are some medical procedures and treatments that you would get for your condition whether you were in this study or not, such as clinic visits, blood draws, or treatment with Letrozole. You and/or your health insurance may be billed for the costs of medical care during this study, if these expenses would have happened even if you were not in the study, or if your insurance agrees in advance to pay. If you have health insurance, the cost of these services will be billed to your insurance company. If your insurance does not cover these costs, or you do not have insurance, these costs will be your responsibility.

Is my participation voluntary?

Taking part in this study is voluntary. You have the right to choose not to take part in this study. If you choose to take part, you have the right to stop at any time. If you refuse or decide to withdraw later, you will not lose any benefits or rights to which you are entitled.

If you leave this study, you will still receive your normal medical care. The only medical care that you will lose is the medical care you are getting as part of this study.

If there are any new findings during the study that may affect whether you want to continue to take part, you will be told about them.

Can I be removed from this study?

The study doctor may decide to stop your participation without your permission if the study doctor thinks that being in the study may cause you harm, or for any other reason. Also, the sponsor may stop the study at any time.

What happens if I am injured or hurt during the study?

If you have an injury while you are in this study, you should call Elena Shagisultanova, MD, PhD immediately. Her phone number is 303-724-0083. In an emergency, call 9-1-1.

We will arrange to get you medical care if you have an injury that is caused by this research. However, you or your insurance company will have to pay for that care.

Who do I call if I have questions?

The researcher carrying out this study is Elena Shagisultanova, MD, PhD. You may ask any questions you have now. If you have questions, concerns, or complaints later, you may call Elena Shagisultanova, MD, PhD at 303-724-0083. You will be given a copy of this form to keep.

You may have questions about your rights as someone in this study. You can call Elena Shagisultanova, MD, PhD with questions. You can also call the responsible Institutional Review Board (COMIRB). You can call them at 303-724-1055.

A description of this clinical trial will be available on http://www.Clinical Trials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

Optional Consent for Research Study Procedures

Here are the optional parts of this study. ***Remember, no matter what you decide to do about this optional part of the study, you may still take part in the main study.*** If you decide to withdraw your consent for the optional parts, you can continue to take part in the main study, unless you withdraw your consent for the main study as well.

Following each optional procedure is a statement asking if you want to participate in the optional procedure. Please read the statement and think about your choice. After reading the sentence, please check “Yes” or “No” and initial next to your choice. If you have any questions, please talk to your doctor or the study team member.

Optional consent for biopsies

Please note that the Optional Tumor Biopsies part of the study is not a requirement of the main study. You can still be in the main study if you do not provide the optional tumor biopsies noted in this section. You can decide at any point not to participate in the optional tumor biopsies.

As part of this study you are being asked if you would like to participate in **optional** tumor biopsies. If you have a tumor from which a core or punch biopsy can be obtained, and you agree to the procedure, a small sample of your tumor tissue will be taken at the following time points:

- Baseline
- Anytime during Cycle 2
- End of treatment

I give my permission for a biopsy of my tumor to be taken at the time points listed above and stored in a central tissue bank at the University of Colorado Denver for this research.

☐ Yes ☐ No _____Initials

Optional consent for data and specimen banking for future research

Dr. Shagisultanova would like to keep some of the data, blood and tissue that is taken during the study but is not used for other tests. If you agree, the data and samples will be kept and may be used in future research to learn more about breast cancer. The research that is done with your data and samples is not designed to specifically help you. It might help people who have breast cancer and other diseases in the future. Reports about research done with your data and samples will not be given to you or your doctor. These reports will not be put in your health records. The research using your data and samples will not affect your care.

The choice to let Dr. Shagisultanova keep the data and samples for future research is up to you. No matter what you decide to do, it will not affect the care that you will receive as part of the study. If you decide now that your data and samples can be kept for research, you can change your mind at any time and contact your study doctor to let him or her know that you do not want Dr. Shagisultanova to use your data and samples any longer, and they will no longer be used for research. Otherwise, they may be kept until they are used up, or until Dr. Shagisultanova decides to destroy them.

When your data and samples are given to other researchers in the future, Dr. Shagisultanova will not give them your name, address, phone number or any other information that will let the researchers know who you are.

Sometimes data and samples are used for genetic research (about diseases that are passed on in families). Even if your data and samples are used for this kind of research, the results will not be told to you and will not be put in your health records. Your data and samples will only be used for research and will not be sold. The research done with your data and samples may help to develop new products in the future, but there is no plan for you to be paid.

The possible benefits of research from your data and samples include learning more about what causes breast cancer and other diseases, how to prevent them and how to treat them. The greatest risk to you is the release of your private information. Dr. Shagisultanova will protect your records so that your name, address and phone number will be kept private. The chance that this information will be given to someone else is very small. There will be no cost to you for any data or sample collection and storage by Dr. Shagisultanova and the University of Colorado Denver.

Please read each sentence below and think about your choice. After reading each sentence, circle “yes” or “no.” If you have questions, please talk to your doctor or nurse. Remember, no matter what you decide to do about the storage and future use of your data and samples, you may still take part in the study.

I give my permission for my data, blood and tissue to be stored in a central tissue bank at the University of Colorado Denver for future use by the study investigators:

1. I give my permission for my data, blood and tissue samples to be kept by Dr. Shagisultanova for use in future research to learn more about how to prevent, detect, or treat breast cancer.

   ☐ Yes ☐ No _____Initials

2. I give my permission for my data, blood and tissue samples to be used for research about other health problems (for example: causes of heart disease, osteoporosis, diabetes, etc.).

   ☐ Yes ☐ No _____Initials

3. I give my permission for my study doctor (or someone he or she chooses) to contact me in the future to ask me to take part in more research.

☐ Yes ☐ No _____Initials

Who will see my research information?

The University of Colorado Denver (UCD) and its affiliated hospital(s) have rules to protect information about you. Federal and state laws including the Health Insurance Portability and Accountability Act (HIPAA) also protect your privacy. This part of the consent form tells you what information about you may be collected in this study and who might see or use it.

The institutions involved in this study include:

- University of Colorado Denver
- University of Colorado Hospital

We cannot do this study without your permission to see, use and give out your information. You do not have to give us this permission. If you do not, then you may not join this study.

We will see, use and disclose your information only as described in this form and in our Notice of Privacy Practices; however, people outside the UCD and its affiliate hospitals may not be covered by this obligation.

We will do everything we can to maintain the confidentiality of your personal information but confidentiality cannot be guaranteed.

The use and disclosure of your information has no time limit. You can cancel your permission to use and disclose your information at any time by writing to the study's Principal Investigator (PI), at the name and address listed below. If you do cancel your permission to use and disclose your information, your part in this study will end and no further information about you will be collected. Your cancellation would not affect information already collected in this study.

Study Principal Investigator (PI) address:

Elena Shagisultanova, MD, PhD
12801 East 17th Avenue
Mail Stop 8117, Room 8101A
Aurora, CO 80045

Both the research records that identify you and the consent form signed by you may be looked at by others who have a legal right to see that information, such as:

- Federal offices such as the Food and Drug Administration (FDA) and the Office of Human Research Protections (OHRP) that protect research subjects like you.
- People at the Colorado Multiple Institutional Review Board (COMIRB).
- The study doctor and the rest of the study team.
- Pfizer, Inc., the company providing funding support and the study drug palbociclib.
- Seattle Genetics, Inc., who is providing the study drug tucatinib.
- Officials at the institution where the research is conducted and officials at other institutions involved in this study who are in charge of making sure that we follow all of the rules for research.
- Criterium, Inc., a Contract Research Organization who is assisting with the management and monitoring of this study

We might talk about this research study at meetings. We might also print the results of this research study in relevant journals, but we will always keep the names of the research subjects, like you, private.

You have the right to request access to your personal health information from the Investigator. To ensure proper evaluation of test results, your access to these study results may not be allowed until after the study is completed.

The investigator (or staff acting on behalf of the investigator) will use your information for the research outlined in this consent form. They will also make all or some of the following health information about you collected in this study available to:

- Other approved participating sites
- External collaborating institutions

Some of the research procedures involve genetic testing or the use of your genetic information. Some of your specimens and de-identified results of tumor molecular analysis (data on genes and proteins in your tumor), and/ or de-identified health information might also be placed into one or more external publicly-accessible scientific databases. For example, the National Institutes of Health (an agency of the federal government) maintains a genetic database called “The Database of Genotypes and Phenotypes” (dbGaP). The information that could directly identify you (such as your name, address, or social security number) will never be placed into these external databases. A researcher who wants to study de-identified information from these databases must have an approved study and work with the group overseeing the database to obtain the information.

A Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways:

- Health insurance companies and group health plans may not request your genetic information that we get from this research.
- Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums.
- Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment.

All health insurance companies and group health plans must follow this law by May 21, 2010. All employers with 15 or more employees must follow this law as of November 21, 2009.

Be aware that this Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

Information about you that will be seen, collected, used, and disclosed in this study:

- Name and Demographic Information (age, sex, ethnicity, address, phone number, etc.
- Portions of your previous and current Medical Records that are relevant to this study, including but not limited to Diagnosis(es), History and Physical, laboratory or tissue studies, radiology studies, procedure results
- Research Visit and Research Test records
- Tissue samples and the data with the samples.
- Billing or financial information

What happens to Data, Tissue, Blood and Specimens that are collected in this study?

Scientists at the University of Colorado Denver and the hospitals involved in this study work to find the causes and cures of disease. The data, tissue, blood and specimens collected from you during this study are important to this study and to future research. If you join this study:

- The data, tissue, blood, or other specimens given by you to the investigators for this research no longer belong to you.

- Both the investigators and any sponsor of this research may study your data, tissue, blood, or other specimens collected from you.
- If data, tissue, blood, or other specimens are in a form that identifies you, UCD or the hospitals involved in this study may use them for future research only with your consent or Institutional Review Board (IRB) approval.
- Any product or idea created by the researchers working on this study will not belong to you.
- There is no plan for you to receive any financial benefit from the creation, use or sale of such a product or idea.

HIPAA Authorization for Optional Additional Study Procedures

In this form, you were given the option to agree to additional, optional research procedures. You must also give us your permission, under HIPAA rules, to use and disclose the information collected from these optional procedures, as described above.

If you decline to give us permission to use and disclose your information, you cannot take part in these optional procedures, but you can still participate in the main study. Please initial next to your choice:

_____ I give permission for my information, from the optional procedures I have agreed to above, to be used and disclosed as described in this section.

_____ I **do not** give permission for my information for any optional procedures to be used and disclosed; I understand that I will not participate in any optional procedures.

Agreement to be in this study and use my data

I have read this paper about the study or it was read to me. I understand the possible risks and benefits of this study. I understand and authorize the access, use and disclosure of my information as stated in this form. I know that being in this study is voluntary. I choose to be in this study: I will get a signed and dated copy of this consent form.

Signature: _____ Date: _____

Print Name: _____

Consent form explained by: _____ Date: _____

Print Name: _____

Use the following only if applicable.

Witness Signature: _____ Date: _____

Witness Print Name: _____

Witness of Signature: ☐
Witness of consent process: ☐

Principal Investigator: **Elena Shagisultanova, MD, PhD**

COMIRB No: **16-1661**

CRITERIUM No: **17BR01**

Version Date: **March 19, 2020**

Study Title: **PHASE IB/II OPEN-LABEL SINGLE ARM STUDY TO EVALUATE SAFETY AND EFFICACY OF TUCATINIB IN COMBINATION WITH LETROZOLE AND PALBOCICLIB IN SUBJECTS WITH HORMONE RECEPTOR POSITIVE AND HER2-POSITIVE METASTATIC BREAST CANCER**

You are being asked to be in a research study. This form provides you with information about the study. A member of the research team will describe this study to you and answer all of your questions. Please read the information below and ask questions about anything you do not understand before deciding whether or not to take part.

Why is this study being done?

This study plans to learn more about the combination therapy of tucatinib with palbociclib and letrozole and how these drugs work together to treat your type of cancer. Letrozole has been approved as standard treatment for metastatic breast cancer by the U.S. Food and Drug Administration (FDA). Palbociclib has been approved by the FDA for treatment of hormone receptor positive (HR+) metastatic breast cancer, but has not yet been approved for patients with HR+ and HER2+ metastatic breast cancer. Tucatinib has not been approved for combination treatment of Palbociclib and letrozole in metastatic breast cancer patients. However, Tucatinib has been approved for combination treatment with trastuzumab and capecitabine for patients with advanced unresectable or metastatic HER2+ breast cancer. This is considered an “Investigational” study because this combination of drugs has not yet been approved by the FDA. The purpose of this study is to evaluate the safety and how well the study drugs are tolerated when given in combination.

You are being asked to be in this research study because you have been diagnosed with HR+/HER2+ metastatic breast cancer.

Throughout the rest of this form, when these drugs are referenced by themselves they will be called tucatinib, palbociclib, and letrozole, but when referenced together or in combination they will be called the **“study drugs”.**

Other people in this study

This study has two parts. The first part is a dose de-escalation, also called Phase Ib. The second part is an expansion, also called Phase II. These parts will be described in the next section of this consent form.

Up to 25 people from around the country will participate in the Phase Ib part of this study.

Up to 50 people from around the country will participate in both parts of the study.

What happens if I join this study?

If you join the study, you will be asked to sign this consent form. You will be given a copy to keep and the original form will be kept at the clinic. You can withdraw from the study at any time and without giving a reason. This will not affect the standard medical care you receive.

The two parts to this study are:

Part I – Phase Ib
Dose de-escalation group (cohort) - The goal of this part of the study is to see how safe tucatinib is when taken in combination with palbociclib and letrozole. In this part of the study, the tucatinib and palbocilib doses can be changed for safety. You will continue to take the study drugs as long as it is safe and effective for you to do so.

Part II – Phase II
Expansion group (cohort) – In this part of the study we will use the doses of the study drugs determined from Part I. We will further study how effective this combination of study drugs may be in treating HR+/HER2+ metastatic breast cancer.

By signing this consent form, you will be taking part in Phase Ib of this study, which has three (3) sections:

1. Before starting the study (Screening)
2. During the study (Treatment)
3. Completion of study (End of Treatment and Follow-up)

There are also optional parts of this study. These optional procedures are voluntary and are not required. You can still take part in the main study if you choose not to take part in the optional study procedures. You will be given the choice later in this consent form to decide if you would like to take part in these optional procedures.

This next section is an overview of what will be expected of you, and what you can expect if you take part in this study.

Study Procedures:

Below are the study procedures and schedule of events (when each procedure will take place) for the dose de-escalation group (Part I) of this study. Some procedures you receive while taking part in this study are "standard of care procedures" for treatment of your disease. If you have had some of these procedures recently, they may not need to be repeated. Some procedures are required only for this research study and will be called "research" procedures. The time points when these study procedures will take place are specified in the next section called "Study Visits".

- **Informed Consent**
  This informed consent form will be discussed with you and you will be given a copy of this document. If you join the study, you will be asked to sign this consent form before you receive any study related tests or procedures.

- **Medical and Cancer History**
  Before you start the study, we will record your date of birth, race, ethnicity, and complete medical history. This medical history will look at the background and history of your cancer and any treatments you have received for your disease.

- **Physical Examination**
  A physical examination will be completed as part of your standard of care at screening and throughout the duration of the study. After you join the study, we will assess if the study drugs are affecting your body functions including lungs, heart, abdomen, extremities, skin, head (eyes, ears, nose, hair, etc.) and your neurological function.

- **Tumor Tissue Samples**
  - Archived Tissue. If you had surgery for your cancer in the past, you must agree to allow us to contact the institution where you had your surgery and ask them to send us a portion of your tumor tissue that they have stored so we may use it for this research.
  - Tumor tissue biomarker testing during the study
    We will ask you to allow us to take a fresh biopsy of your tumor tissue and keep it for research. There are three optional tumor biopsies in this study:

    (1) prior to the start of treatment,
    (2) after completion of the first 28 days of treatment, and
    (3) at the end of the study.

    This is a voluntary part of this study, and you will be given the option later in this consent form to take part, or not take part, in this optional procedure.

- **Blood and Urine Samples**
  These tests are sometimes called safety labs so the study doctor can be sure it is safe for you to take part in this study and to be given the study drugs. These tests will include some standard of care tests and some research tests:
  - Pregnancy test: Women who are able to become pregnant will be given either a urine or a blood pregnancy test. A positive pregnancy test prior to being given the study drugs will exclude you from starting or continuing to take part in the study.
  - Complete blood count (CBC)
  - Comprehensive metabolic panel (CMP)
  - Pharmacokinetic (PK) blood test. This is a research related test that will allow us to see how long the study drugs remain in your body.
  - Blood clotting tests (PT/INR, and PTT)
  - Biomarker blood test. This research test will look at how and if your cancer is responding to the treatment.

  Your doctor may order additional blood tests that he/she feels are needed to plan treatment, dose modification, or further evaluations.

- **Electrocardiogram (ECG)**
  This is a noninvasive procedure that records the electrical activity of the heart. Electrodes are placed on the skin of the chest and connected in a specific order to a machine. Output usually appears on a long scroll of paper that displays a printed graph. This will check the electrical activity of your heart.

- **Echocardiogram (ECHO) or Multigated Acquisition (MUGA) Scan**
  This is a noninvasive scan of the heart using sound waves. This test will be used to see how well your heart pumps blood.

- **Vital Signs**
  We will take your blood pressure, heart rate, respiratory rate, body temperature and weight. Height will be measured only during screening.

- **Performance Status**
  We will assess how well you are performing your daily activities.

- **Other Medications**
  Your study doctor will let you know which other medications you can and cannot take while taking part in this study. You will need to check with the study doctor

before taking any new medications. From the time you first receive the study drugs through 30 days after the last dose, we will record other medications you may be taking.

- **Review of the Adverse Events (AEs)**
  Some risks have been identified because of the disease process or through use of the study drugs. These are commonly called side effects and will be followed very closely by your doctor and the study staff. More information about these will be provided in the Risk section of this consent form.

Study Visits:

1. SCREENING

After signing this consent form you will have the following done to see if you can be in this study:

- Review your medical and cancer history
- ECHO or MUGA scan of your heart
- Physical exam
- Vital signs
- Performance status
- Blood tests:
  - Complete blood count (CBC) including types – white cells, red cells and platelets (differential) and comprehensive metabolic panel (CMP)
  - Blood clotting (PT/INR and PTT) – **research**
  - Pregnancy (women of childbearing potential)
  - Biomarker blood sample – **research**
- Review of medications you are taking
- ECG – **research**
- Imaging studies to document sites of disease and assess tumor burden. This may include diagnostic quality CT scan and bone scan to document the locations of the cancer in your body. It may also include a brain MRI, if one is necessary.
- Tumor tissue collections of archived biopsies – **research**
- Tumor tissue biopsy - **optional research procedure**
  You do not have to have this procedure if you do not want to. You will be given the choice later in this consent form to decide if you want to take part in this part of the study.

If the procedures above show that you are eligible to participate in the study, you will undergo the following procedures while on treatment.

2. TREATMENT

Cycle 1 Day 1

- Review of AEs
- Review of medications you are taking
- Physical examination
- Vital signs
- Performance status
- Blood tests – CBC with differential and CMP
- Begin treatment with the study drugs once instructed at clinic visit – **research**
- You will be given a drug diary to complete – **research**

Cycle 1 Day 15

- Review of AEs
- Review of medications you are taking
- Vital signs
- Blood tests – CBC with differential
- Blood samples for PK analysis (described above) will be taken before you receive tucatinib and palbociclib, and after receiving these study drugs at 30 minutes, and 1, 2, 3, 4 and 6 hours after receiving the dose – **research**
- Review drug diary – **research**
- Continue treatment with the study drugs once instructed at clinic visit – **research**

Cycle 2 Day 1

- Review of AEs
- Review of medications you are taking
- Physical examination
- Vital signs
- Performance status
- Blood tests
    - CBC with differential and CMP
    - Blood samples for PK analysis will be taken before you receive tucatinib and palbociclib – **research**
    - Biomarker blood sample – **research**
- Review drug diary from previous cycle – **research**

- Treatment with the study drugs once instructed at clinic visit – **research**
- You will be given a new drug diary to complete – **research**
- Tumor tissue biopsy - **optional research procedure**
  This procedure can be performed at any time during cycle 2. You do not have to have this procedure if you do not want to. You will be given the choice later in this consent form to decide if you want to take part in this part of the study.

Cycle 2 Day 15

- Review of AEs
- Review of medications you are taking
- Vital signs
- Blood tests – CBC with differential
- Review drug diary – **research**
- Continue treatment with the study drugs once instructed at clinic visit – **research**

Cycle 3+ Day 1

- Review of AEs
- Review of medications you are taking
- Physical examination
- Vital signs
- Performance status
- Blood tests – CBC with differential and CMP
- Review drug compliance from previous cycle and drug diary
- Administer treatment with the study drugs once instructed at clinic visit
- You will be given a new drug diary to complete

Additional Study Procedures

While you are on this study, you will also have the following procedures done at regular time points.

- *Once every 8 weeks for the first 24 weeks (6 cycles), then once every 12 weeks:* Imaging studies to document sites of disease and assess tumor burden. This may include diagnostic quality CT scan and bone scan to document the locations of the cancer in your body. It may also include a brain MRI, if one is necessary.
- *Once every 12 weeks:* ECHO or MUGA scan

3. COMPLETION OF STUDY – (End of treatment and Follow-up)

You will need to return to clinic for a follow-up study visit if your disease gets worse, if you decide to withdraw from the study, or if you start a different anticancer therapy.

The following will be performed if not already performed in the prior week.

- Review of AEs
- Review of medications you are taking
- Review drug compliance from previous cycle and drug diary
- Vital signs
- Physical exam
- Performance status
- Blood tests
    - CBC with differential and CMP
    - Additional biomarker blood sample – **research**
- Imaging studies to document sites of disease and assess tumor burden. This may include diagnostic quality CT scan and bone scan to document the locations of the cancer in your body. It may also include a brain MRI, if one is necessary.
- Tumor tissue biopsy - **optional research procedure**
  You do not have to have this procedure if you do not want to. You will be given the choice later in this consent form to decide if you want to take part in this part of the study.

You will also need to return to the clinic for an additional safety follow-up visit in about 30 days after you stop treatment with the study drugs. This visit will include:

- Review of AEs
- Review of medications you are taking
- Vital signs
- Physical exam
- Performance status
- Blood tests – CBC with differential and CMP

How long will I be on the study?

You may take part in this study for as long as you tolerate the study tests, procedures, and treatments and your disease is stable or improving. You will continue to receive treatment with the study drugs until your disease progresses or you have an unacceptable drug-related side effect.

What are the possible discomforts or risks?

As with any study drug, side effects may occur when taking this study drug. While taking part in this study, and being treated with the study drugs, you will be watched carefully for any side effects. Some side effects may go away after you stop taking the study drug. Some side effects can be long lasting and may never go away or may even lead to death.

You should talk to your study doctor about any side effects or discomfort you may have. The study doctor may give you some medicine that will help with some side effects. The study doctor may also interrupt or discontinue the study drug.

You will be notified by your study doctor of any new side effects seen in other patients that occur during the time you are on the study. This may affect you wanting to continue in this research study.

You should avoid drinking grapefruit juice in large amounts (>34 oz. or 1 L) and eating cranberries or drinking cranberry juice while participating in this study as it could affect how your body metabolizes the drugs.

You may experience risks or discomforts when taking part in this study, which include some that are common such as low blood cell counts, infections, gastrointestinal (stomach and intestines) symptoms, fever, and fatigue, some that are rare, such as life-threatening infections and sepsis, and some that are unknown at this time. This is not a complete list of risks or discomforts. A comprehensive list of risks and discomforts is provided later in this consent document.

Palbociclib has been given to approximately 1,674 patients with breast cancer who received palbociclib together with hormonal treatment in Pfizer-sponsored clinical trials.

Serious and life-threatening infections have been observed in some patients treated with palbociclib.

In a rat study where palbociclib was administered for the lifespan of the rat, microglial cell (a type of cell located in the central nervous system) tumors were seen at blood

concentrations higher than those used to treat humans. It is currently unknown what these findings observed only in male rats means for patients treated with palbociclib over time.

Based on animal studies and prior human studies with the study drugs, and/or other studies with similar types of drugs, side effects or discomforts you may experience while in this study include**:**

Risks of the Study Drugs:

Tucatinib

Common - in more than 20 out of 100 people

- Diarrhea
- Dizziness
- Fatigue
- Nausea
- Rash
- Weakness

Less Common - in 1 to 20 people out of 100 people

- Pain in the abdomen (belly pain)
- Alkaline phosphatase elevation
- Constipation
- Cough
- Creatinine increase (decreased kidney function)
- Headache
- Pain in arms or legs
- Chest pain
- Muscle pain
- Shortness of breath/difficulty breathing
- Potentially life-threatening abnormal liver function tests (hepatitis)
- Anemia (low hemoglobin), which may make you feel tired or weak
- Decreased appetite
- Low sodium, potassium, magnesium and phosphate levels, or level of other electrolytes in your body
- Bilirubin elevation (break down of red blood cells)
- Leg swelling
- Vomiting
- Infection in the nose, sinuses or throat (upper respiratory tract infection). You may have fever, pain, or a hard time breathing.
- Heavy sweating while sleeping (night sweats)

- Back pain
- Infection that could cause frequent and painful urination (urinary tract infection)
-

Rare - in fewer than 1 out of 100 people

- Weakness of the heart muscle (heart failure)
- EKG changes (change in your heart's electrical activity)/QT prolongation (takes heart longer than normal to recharge during beats)/irregular heartbeat/palpitations
- Dehydration

Most Important Side Effects -

- **Liver Function Problems**
  Some participants who took tucatinib had problems with their liver. Your doctor will run tests to check that your liver is working properly during your treatment with tucatinib. Your doctor may change your tucatinib dose based on the test results.

- **Diarrhea**
  Tucatinib can cause severe watery poop (diarrhea). If you have watery poop, tell your doctor **right away**. Your doctor may change your tucatinib dose if you have watery poop.

- **Kidney Function Problems**
  Some participants who got tucatinib had higher creatinine levels. This could mean that there are problems with your kidneys. If this happens, it's usually within the first treatment cycle. Participants who have had increased levels of creatinine had these levels stay increased while they were getting treated. The levels went back down after treatment stopped.

- **Interactions with Other Medications**
  Tucatinib may change how your body reacts to some other drugs, which could stay in your body longer than usual. While you are taking part in this study, you might need to stop some medications or replace them with other medications. If you can't stop taking these medications, your doctor might need to change how much you take. Your doctor might also need to watch your health more closely while you are taking part in this study.

Palbociclib

More Common - in more than 30 out of 100 people

- Decrease in neutrophil blood cells (may increase the risk for infection)
- Decrease in white blood cell count (infection fighting cells)
- Infections, including serious bacterial or viral infections or reactivation of previous infections
- Feeling tired or fatigued

Common - in 10 to 30 people out of 100 people

- Anemia (low hemoglobin), which may make you feel tired or weak
- Low platelet count, which may increase your risk of bleeding or bruising
- Inflammation or sores of the mouth or throat, which may make it difficult to talk, eat, or swallow
- Diarrhea
- Constipation
- Nausea
- Vomiting
- Joint pain
- Back pain
- Pain in the hands and feet
- Hair loss
- Rash
- Cough
- Shortness of breath
- Headache
- Decreased appetite
- Hot Flush
- Dizziness
- Inability to sleep (insomnia)
- Common Cold
- Fever

Less Common - in 5 to less than 10 people out of 100 people

- Abdominal pain
- Indigestion
- Dry mouth
- Weakness (asthenia)
- Swelling of the hands and feet

- Irritation or sores in the lining of hollow organs like mouth, throat, stomach, or bowels
- Pain
- Influenza- (flu-) like illness
- Muscle pain
- Pain in the muscles and bone including around the chest and neck
- Muscle cramps
- Increases in blood liver markers that may indicate liver damage, including potentially life threatening liver damage
- Dry skin
- Itching
- Mouth or throat pain
- Nosebleeds
- Impaired sense of taste
- Depression
- Falls
- Anxiety
- Increased blood pressure
- Heartburn (acid reflux)
- Increased creatinine level that could indicate abnormal kidney function

Rare - in less than 5 out of 100 people

- Fever associated with a dangerously low level of a type of white blood cell counts (neutrophils)
- Blurred vision
- Dry eyes or watery eyes
- Interstitial lung disease / pneumonitis (an inflammation of the lungs which can cause cough and shortness of breath)

Letrozole

Common – in more than 20 out of 100 people

- Hot flashes or feeling flushed
- Joint pain or arthritis
- Mood swings
- Pain in the muscles
- Dryness in the vaginal area

Less Common – in 1 to 20 people out of 100 people

- Dizziness

- Fatigue
- Headache
- Difficulty sleeping
- Elevated blood pressure
- Elevated blood sugar
- Elevated cholesterol
- Nausea or vomiting
- Weight gain
- Night sweats
- Loss of bone mineral density, bone fracture
- Kidney and urinary bladder infections
- Vaginal bleeding

Rare - in less than 1 out of 100 people

- Constipation

Risks of Having Blood Taken

In this study, we will need to get about 30 tablespoons of blood from you over the course of the study for research. This is in addition to the standard of care blood tests. We will get blood by putting a needle into one of your veins and letting the blood flow into a vacuum tube. You may feel some pain when the needle goes into your vein. A day or two later, you may have a small bruise where the needle went under the skin.

Risks of biopsy

In this study, you will have an option to give up to 3 biopsies for research. There are some risks to taking a biopsy. There is a small chance that you could get an infection where the needle goes in. You may also experience redness, swelling, minor bleeding or bruising at the site where the cut was made or the needle inserted. You may experience mild to moderate pain at the site of the needle puncture. There is also a small chance that you could have an allergic reaction to the numbing medicine. After your skin heals up, you may have a small scar where we take the samples.

Archival tumor tissue

A section will be taken from tumor tissue samples you may have had in the past. Since this has already been removed from you, there are no additional risks to you.

Risks of having an ECG

An electrocardiogram (ECG) is a test that records the electrical activity of the heart. Skin irritation is rare but could occur during an ECG from the electrodes or gel that is used.

Risks of Having an IV Inserted in Your Vein

In this study, we may need to insert a needle, connected to a plastic tube, into a vein in your arm. You will feel some pain when we first insert the tube into your vein. You may have some redness, swelling, or bruising where the tube goes under your skin. In some cases, this type of tube can cause an infection where it goes under the skin. In rare cases, it can cause a blood clot in the vein. You may have this tube inserted for about 6 hours for the PK draw on Cycle 1 Day 15.

Risks Associated with Pregnancy

The use of the study drugs in pregnant females and nursing mothers has not been studied. The effects of the study drugs on human eggs and sperm has not been studied. The risks to a human fetus are unknown.

However, animal testing has shown embryo-fetal toxicity that could be a potential risk in humans for all the study drugs (tucatinib, palbociclib, and letrozole) in this study.

All female and male subjects of childbearing potential must use an acceptable form of birth control while on this study, as females must not become pregnant while taking the study drugs. Before you begin study treatment, your doctor will discuss acceptable forms of birth control with you.

Tucatinib, Palbociclib, or letrozole could hurt or kill a baby growing in the womb. You should not try to get pregnant or get your partner pregnant while you are on this clinical trial. An effective method of birth control should be used for up to 30 days after the end of this study.

If you are pregnant or breastfeeding, you cannot be in this study. We will remove anyone who becomes pregnant while on study from the study treatment immediately.

Additionally, tucatinib, palbociclib, or letrozole could hurt a breastfeeding baby. You must not breastfeed during the study and you should wait to breastfeed for up to 30 days after the end of treatment.

You should tell your study doctor **right away** if:

- you become pregnant while you are in this study
- you think that you are pregnant while taking part in this study
- you or your partner become pregnant within 30 days after being treated with tucatinib, palbociclib, and letrozole.

Birth Control Requirements

If you can get pregnant, you must use a safe birth control method while you are taking part in this study. Safe birth control methods fail less than 1% of the time. This means that if 100 people used this birth control for a whole year, at most 1 person would get pregnant. You must use safe birth control methods while you take part in the study and keep using them for 30 days after stopping all study treatment. If you do not use safe birth control methods while you take part in the study, we might decide that you cannot take part in the study anymore.

The safe birth control methods that you can use are:

- intrauterine device (IUD) like Paragard
- tubal ligation for females (“tubes tied”)
- condoms/vaginal diaphragm
- vasectomy for males

Talk with your doctor about your birth control options. You may also choose to not be sexually active (complete abstinence) while you take part in the study.

If you are able to get pregnant, you must use a barrier method such as a male or female condom when you are taking part in the study. You must keep using a barrier method for 30 days after finishing treatment.

Fertility Risks

It is not known if patients in this trial will have problems getting pregnant after taking tucatinib, palbociclib, or letrozole.

Risk of Loss of Confidentiality

There is a risk that people outside of the research team will see your research information. We will do all that we can to protect your information, but it cannot be guaranteed.

The study may include risks that are unknown at this time.

What are the possible benefits of the study?

This study is designed for the researcher to learn more about the effects of the study drug on your disease. However, there is no guarantee that your health will improve if you join this study. Also, there could be risks to being in this study. If there are risks, these are described in the section describing the discomforts or risks.

Are there alternative treatments?

There may be other ways of treating your (HR+/HER2+) metastatic breast cancer.

These other ways include:

- You may choose to receive treatment with another experimental therapy.
- You may choose to receive treatment with another approved therapy.
  Approved therapies for patients with HR+/HER2+ metastatic breast cancer include:
  (1) anti-endocrine agents (tamoxifen, fulvestrant, anastrozole, letrozole, exemestane)
  (2) combination of anti-endocrine agents and HER2-targeted agents (trastuzumab, pertuzumab, lapatinib)
  (3) combination of chemotherapy and HER2-targeted agents
  (4) antibody-drug conjugate TDM-1
- You may choose to receive comfort/palliative care.
- You could also choose to get no treatment at all.

You should talk to your doctor about your choices. Make sure you understand all of your choices before you decide to take part in this study. You may leave this study and still have these other choices available to you.

Who is paying for this study?

This research is being conducted by Elena Shagisultanova, MD, PhD, with funding support by Pfizer, Inc., who is also providing the study drug palbociclib. Seattle Genetics will provide the other study drug, tucatinib. You will be required to pay for your treatment with letrozole as part of your standard care.

The sponsor will only pay for procedures not considered standard of care.

Ask your study doctor to discuss any costs that will not be covered by the study. This discussion should include the costs of treating possible side effects. Otherwise, you might have unexpected expenses from being in this study.

Will I be paid for being in the study?

You will not be paid to be in the study.

Will I have to pay for anything?

There are some medical procedures and treatments that you would get for your condition whether you were in this study or not, such as clinic visits, blood draws, or treatment with letrozole. You and/or your health insurance may be billed for the costs of medical care during this study, if these expenses would have happened even if you were not in the study, or if your insurance agrees in advance to pay. If you have health insurance, the cost of these services will be billed to your insurance company. If your insurance does not cover these costs, or you do not have insurance, these costs will be your responsibility.

Is my participation voluntary?

Taking part in this study is voluntary. You have the right to choose not to take part in this study. If you choose to take part, you have the right to stop at any time. If you refuse or decide to withdraw later, you will not lose any benefits or rights to which you are entitled.

If you leave this study, you will still receive your normal medical care. The only medical care that you will lose is the medical care you are getting as part of this study.

If there are any new findings during the study that may affect whether you want to continue to take part, you will be told about them.

Can I be removed from this study?

The study doctor may decide to stop your participation without your permission if the study doctor thinks that being in the study may cause you harm, or for any other reason. Also, the sponsor may stop the study at any time.

What happens if I am injured or hurt during the study?

If you have an injury while you are in this study, you should call Elena Shagisultanova, MD, PhD immediately. Her phone number is 303-724-0083. In an emergency, call 9-1-1.

We will arrange to get you medical care if you have an injury that is caused by this research. However, you or your insurance company will have to pay for that care.

Who do I call if I have questions?

The researcher carrying out this study is Elena Shagisultanova, MD, PhD. You may ask any questions you have now. If you have questions, concerns, or complaints later, you may call Elena Shagisultanova, MD, PhD at 303-724-0083. You will be given a copy of this form to keep.

You may have questions about your rights as someone in this study. You can call Elena Shagisultanova, MD, PhD with questions. You can also call the responsible Institutional Review Board (COMIRB). You can call them at 303-724-1055.

A description of this clinical trial will be available on http://www.Clinical Trials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

Optional Consent for Research Study Procedures

Here are the optional parts of this study. ***Remember, no matter what you decide to do about this optional part of the study, you may still take part in the main study.*** If you decide to withdraw your consent for the optional parts, you can continue to take part in the main study, unless you withdraw your consent for the main study as well.

Following each optional procedure is a statement asking if you want to participate in the optional procedure. Please read the statement and think about your choice. After reading the sentence, please check "Yes" or "No" and initial next to your choice. If you have any questions, please talk to your doctor or the study team member.

Optional consent for biopsies

Please note that the Optional Tumor Biopsies part of the study is not a requirement of the main study. You can still be in the main study if you do not provide the optional tumor biopsies noted in this section. You can decide at any point not to participate in the optional tumor biopsies.

As part of this study, you are being asked if you would like to participate in **optional** tumor biopsies. If you have a tumor from which a core or punch biopsy can be obtained, and you agree to the procedure, a small sample of your tumor tissue will be taken at the following time points:

- Baseline
- Anytime during Cycle 2
- End of treatment

I give my permission for a biopsy of my tumor to be taken at the time points listed above and stored in a central tissue bank at the University of Colorado Denver for this research.

☐ Yes ☐ No _____Initials

Optional consent for data and specimen banking for future research

Dr. Shagisultanova would like to keep some of the data, blood and tissue that is taken during the study but is not used for other tests. If you agree, the data and samples will be kept and may be used in future research to learn more about breast cancer. The research that is done with your data and samples is not designed to specifically help you. It might help people who have breast cancer and other diseases in the future. Reports about research done with your data and samples will not be given to you or your doctor. These reports will not be put in your health records. The research using your data and samples will not affect your care.

The choice to let Dr. Shagisultanova keep the data and samples for future research is up to you. No matter what you decide to do, it will not affect the care that you will receive as part of the study. If you decide now that your data and samples can be kept for research, you can change your mind at any time and contact your study doctor to let him or her know that you do not want Dr. Shagisultanova to use your data and samples any longer, and they will no longer be used for research. Otherwise, they may be kept until they are used up, or until Dr. Shagisultanova decides to destroy them.

When your data and samples are given to other researchers in the future, Dr. Shagisultanova will not give them your name, address, phone number or any other information that will let the researchers know who you are.

Sometimes data and samples are used for genetic research (about diseases that are passed on in families). Even if your data and samples are used for this kind of research, the results will not be told to you and will not be put in your health records. Your data and samples will only be used for research and will not be sold. The research done with your data and samples may help to develop new products in the future, but there is no plan for you to be paid.

The possible benefits of research from your data and samples include learning more about what causes breast cancer and other diseases, how to prevent them and how to treat them. The greatest risk to you is the release of your private information. Dr. Shagisultanova will protect your records so that your name, address and phone number will be kept private. The chance that this information will be given to someone else is very small. There will be no cost to you for any data or sample collection and storage by Dr. Shagisultanova and the University of Colorado Denver.

Please read each sentence below and think about your choice. After reading each sentence, circle "yes" or "no." If you have questions, please talk to your doctor or nurse. Remember, no matter what you decide to do about the storage and future use of your data and samples, you may still take part in the study.

I give my permission for my data, blood and tissue to be stored in a central tissue bank at the University of Colorado Denver for future use by the study investigators:

1. I give my permission for my data, blood and tissue samples to be kept by Dr. Shagisultanova for use in future research to learn more about how to prevent, detect, or treat breast cancer.

   ☐ Yes ☐ No _____Initials

2. I give my permission for my data, blood and tissue samples to be used for research about other health problems (for example: causes of heart disease, osteoporosis, diabetes, etc.).

   ☐ Yes ☐ No _____Initials

3. I give my permission for my study doctor (or someone he or she chooses) to contact me in the future to ask me to take part in more research.

   ☐ Yes ☐ No _____Initials

Who will see my research information?

The University of Colorado Denver (UCD) and its affiliated hospital(s) have rules to protect information about you. Federal and state laws including the Health Insurance Portability and Accountability Act (HIPAA) also protect your privacy. This part of the consent form tells you what information about you may be collected in this study and who might see or use it.

The institutions involved in this study include:

- University of Colorado Denver
- University of Colorado Hospital

We cannot do this study without your permission to see, use and give out your information. You do not have to give us this permission. If you do not, then you may not join this study.

We will see, use and disclose your information only as described in this form and in our Notice of Privacy Practices; however, people outside the UCD and its affiliate hospitals may not be covered by this obligation.

We will do everything we can to maintain the confidentiality of your personal information but confidentiality cannot be guaranteed.

The use and disclosure of your information has no time limit. You can cancel your permission to use and disclose your information at any time by writing to the study's Principal Investigator (PI), at the name and address listed below. If you do withdraw your permission to use and disclose your information, your part in this study will end and no further information about you will be collected. Your withdrawal would not affect information already collected in this study.

Study Principal Investigator (PI) address:

Elena Shagisultanova, MD, PhD
12801 East 17th Avenue
Mail Stop 8117, Room 8101A
Aurora, CO, 80045

Both the research records that identify you and the consent form signed by you may be looked at by others who have a legal right to see that information, such as:

- Federal offices such as the Food and Drug Administration (FDA) and the Office of Human Research Protections (OHRP) that protect research subjects like you.
- People at the Colorado Multiple Institutional Review Board (COMIRB).
- The study doctor and the rest of the study team.
- Pfizer, Inc., the company providing funding support and the study drug Palbociclib.
- Seattle Genetics Therapeutics, who is providing the study drug Tucatinib.
- Officials at the institution where the research is conducted and officials at other institutions involved in this study who are in charge of making sure that we follow all of the rules for research.

- Criterium, Inc., a Contract Research Organization who is assisting with the management and monitoring of this study

We might talk about this research study at meetings. We might also print the results of this research study in relevant journals. But we will always keep the names of the research subjects, like you, private.

You have the right to request access to your personal health information from the Investigator. To ensure proper evaluation of test results, your access to these study results may not be allowed until after the study is completed.

The investigator (or staff acting on behalf of the investigator) will use your information for the research outlined in this consent form. They will also make all or some of the following health information about you collected in this study available to:

- Other approved participating sites
- External collaborating institutions

Some of the research procedures involve genetic testing or the use of your genetic information. Some of your specimens and de-identified results of tumor molecular analysis (data on genes and proteins in your tumor), and/ or de-identified health information might also be placed into one or more external publicly-accessible scientific databases. For example, the National Institutes of Health (an agency of the federal government) maintains a genetic database called “The Database of Genotypes and Phenotypes” (dbGaP). The information that could directly identify you (such as your name, address, or social security number) will never be placed into these external databases. A researcher who wants to study de-identified information from these databases must have an approved study and work with the group overseeing the database to obtain the information.

A Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways:

- Health insurance companies and group health plans may not request your genetic information that we get from this research.
- Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums.
- Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment.

All health insurance companies and group health plans must follow this law by May 21, 2010. All employers with 15 or more employees must follow this law as of November 21, 2009.

Be aware that this Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

Information about you that will be seen, collected, used and disclosed in this study:

- Name and demographic information (age, sex, ethnicity, address, phone number, etc.
- Portions of your previous and current medical records that are relevant to this study, including but not limited to diagnosis(es), history and physical, laboratory or tissue studies, radiology studies, procedure results.
- Research visit and research test records.
- Tissue samples and the data with the samples.
- Billing or financial information.

What happens to Data, Tissue, Blood and Specimens that are collected in this study?

Scientists at the University of Colorado Denver and the hospitals involved in this study work to find the causes and cures of disease. The data, tissue, blood and specimens collected from you during this study are important to this study and to future research. If you join this study:

- The data, tissue, blood, or other specimens given by you to the investigators for this research no longer belong to you.
- Both the investigators and any sponsor of this research may study your data, tissue, blood, or other specimens collected from you.
- If data, tissue, blood, or other specimens are in a form that identifies you, UCD or the hospitals involved in this study may use them for future research only with your consent or Institutional Review Board (IRB) approval.
- Any product or idea created by the researchers working on this study will not belong to you.
- There is no plan for you to receive any financial benefit from the creation, use or sale of such a product or idea.

HIPAA Authorization for Optional Additional Study Procedures

In this form, you were given the option to agree to additional, optional research procedures. You must also give us your permission, under HIPAA rules, to use and disclose the information collected from these optional procedures, as described above.

If you decline to give us permission to use and disclose your information, you cannot take part in these optional procedures, but you can still participate in the main study. Please initial next to your choice:

_____ I give permission for my information, from the optional procedures I have agreed to above, to be used and disclosed as described in this section.

_____ I **do not** give permission for my information for any optional procedures to be used and disclosed; I understand that I will not participate in any optional procedures.

Agreement to be in this study and use my data

I have read this paper about the study or it was read to me. I understand the possible risks and benefits of this study. I understand and authorize the access, use and disclosure of my information as stated in this form. I know that being in this study is voluntary. I choose to be in this study: I will get a signed and dated copy of this consent form.

Signature: _____ Date: _____

Print Name: _____

Consent form explained by: _____ Date: _____

Print Name: _____

Use the following only if applicable.

Witness Signature: _____ Date: _____

Witness Print Name: _____

Witness of Signature**:** ☐
Witness of consent process: ☐