2018-0287 INTERACT- INTegrated Evaluation of Resistance and Actionability using Circulating Tumor DNA in HR positive metastatic breast cancers

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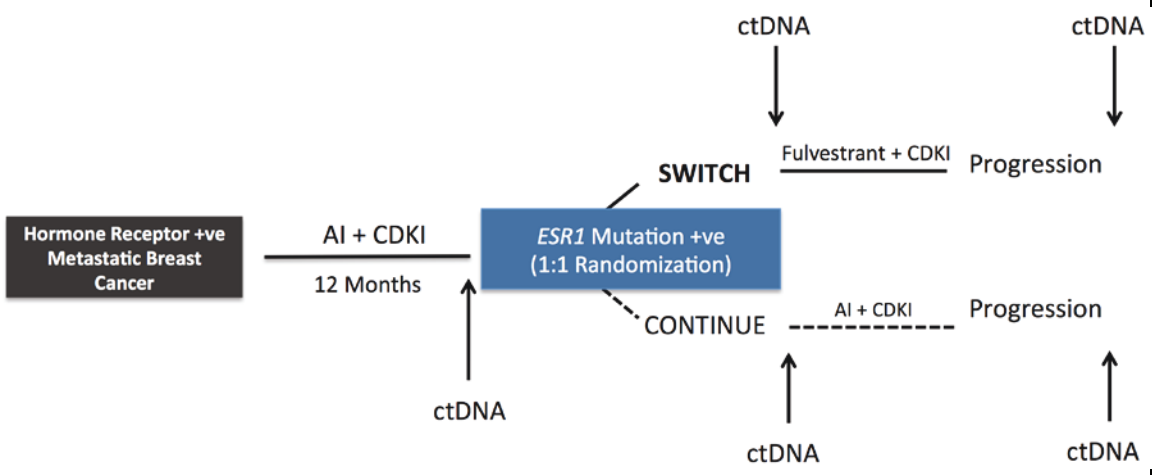
Protocol Type:	**Standard Protocol**
Protocol Phase:	**Phase II**
Version Status:	06

Table of Contents

INTERACT study Schema



Patients with hormone receptor positive MBC who are on first line aromatase inhibitor (letrozole, anastrozole) with CDK inhibitor (palbociclib, ribociclib, abemaciclib) will be screened for *ESR1* mutations. Patients with *ESR1* mutations would be randomized to continuation of treatment versus transition to fulvestrant

1. INTRODUCTION

Breast cancer is the most common invasive cancer in women with an estimated quarter of a million new cases every year in the US[1]. Estrogen positive breast cancers are the most common subtype and contribute to the majority of deaths from breast cancers. While endocrine therapies that target the estrogen-signaling pathway form the backbone of treatment and have shown significant improvement in survival, resistance to therapy is inevitable limiting overall response.

1.1. ESR1 mutations and endocrine resistance

Acquired mutations in *ESR1* (20-30%) have been described in metastatic breast cancer (MBC) patients exposed to estrogen deprivation with aromatase inhibitors (AI) [2-5]. *ESR1* mutations are uncommon in primary breast cancers and rarely observed after adjuvant AI suggesting clonal selection[6]. Though, recent studies have suggested that de novo *ESR1* mutations might be observed at low allele frequencies [7, 8]. Currently the use of AI or AI in combination with cyclin-dependent kinase (CDK) 4/6 inhibitors are recommended for first line treatment of ER positive MBC. Constitutive activation, through mutations in the ligand-binding domain (e.g. Y537S, D538G, S463P), leads to AI resistance and portend a shorter survival [6]. Though, these mutations may exhibit sensitivity to selective estrogen receptor down-regulators (SERD) (e.g. fulvestrant) that target mutant ER for proteasomal degradation[9]. In the BOLERO-2 study, which tested the use of exemestane alone or in combination with everolimus in MBC with prior exposure to AI, plasma *ESR1* mutations (D538G and Y537S only) were identified in nearly 30% of patients. While *ESR1* mutations were associated with shorter overall survival compared to wildtype (32 months), Y537S *ESR1* mutations (20 months) were associated with a worse outcome compared to D538G (26 months) suggesting differences in the variants [10]. In SoFEA trial, which compared the activity of fulvestrant versus exemestane against fulvestrant in combination with anastrozole in hormone-receptor (HR) positive metastatic postmenopausal women who had progressed on prior non-steroidal AI, plasma *ESR1* mutations were observed in nearly 40% of patients. In PALOMA-3 study, which evaluated the addition of CDK 4/6-inhibitor palbociclib to fulvestrant, approximately 25% of patients were found to harbor *ESR1* mutations in plasma, which did not appear to be predictive of palbociclib response. The lower prevalence of *ESR1* mutations in PALOMA3 likely reflects the inclusion of patients with primary resistance to endocrine therapy and prior tamoxifen exposure.

1.2. Circulating Tumor DNA

Improved understanding of tumor evolution and resistance has helped to underscore the importance of repeat biopsies. A major barrier to study genomic evolution and mechanisms of resistance is the challenge associated with obtaining serial tumor biopsies. Depending on the organ, biopsies are associated with procedural complications such as bleeding, pneumothorax, and infections. In a prospective study evaluating the use of biopsies in advanced breast cancers, only about 80% of patients undergoing biopsy had sufficient tissue for receptor analysis [11]. Moreover, more than 35% of biopsies from bone, commonest site of metastasis in hormone receptor positive breast cancers, had insufficient sample yield. Also, radiological assessment has limited sensitivity and cannot discern between cancer cells that are viable vs. non-viable or non-cancer cells such as fibroblasts.

Analyses of circulating tumor DNA (ctDNA) offer a minimally invasive, blood-based, approach to monitor disease and treatment response [12]. Unlike tissue biopsies, ctDNA avoids sampling bias due to intratumor heterogeneity and can provide an objective estimate of tumor burden. Quantitative changes in ctDNA were associated with a better dynamic range and correlation with tumor burden compared to tumor markers in MBC [13]. Assessing residual cancer burden with ctDNA has been shown identify high-risk patients for relapse [14]. ctDNA analysis has been shown to identify *ESR1* mutations not discernable on tissue sampling [15]. Also, a high prevalence of polyclonal *ESR1* mutations has been reported, in patients previously treated with aromatase inhibitor endocrine therapy, suggesting that the complexities of resistant disease due to tumor evolution and heterogeneity is unlikely to be captured by single site tissue biopsy [9].

ctDNA dynamics has been shown to predict responses before such changes are evident on conventional approaches such as imaging. Thus, early ctDNA dynamics can act as a surrogate for assessment of

treatment efficacy with ability to transition from an ineffective therapy to a potentially active one. Furthermore, mutational allele frequency (MAF) represent tumor clones harboring a mutation, thus providing an indicator of tumor burden. Serial monitoring of MAF with ctDNA in patients treated with targeted therapies has been shown to be associated with time to radiologic disease progression [16]. Clatot et al. retrospectively analyzed *ESR1* mutations in ctDNA in patients who were treated with AI for MBC as first line [17]. *ESR1* mutations were identified in 31% of patients at progression with AI. Patients with *ESR1* mutations had worse median PFS (5.9 months) compared to patients without a mutation (7 months). In patients with *ESR1* mutations, 75% of them had detectable circulating mutations before clinical progression. Also, patients presenting with increases in circulating *ESR1* at 3 months after subsequent treatment with prior AI had disease progression. In the FERGI study, patients with radiologic response (complete or partial) demonstrated significant decreases in *ESR1* plasma MAF post treatment with fulvestrant and these were detected early in the course of response. ctDNA analysis of NSCLC patients treated with EGFR inhibitors, showed that EGFR T790M, a common mechanism of secondary resistance ctDNA was detected nearly 2 months prior to clinical progression and that the EGFR T790M ctDNA positive patients had significantly shorter survival [18]. ctDNA velocity with decrease in MAF at 6 weeks were shown to be predictive of response and associated with improved survival to durvalumab in lung cancer [19]. Similarly, in the study evaluating the efficacy of neratinib in advanced breast cancer patients with ERBB2 mutations, decrease in MAF at 4 weeks was predictive of clinical response [20].

ctDNA dynamics of PIK3CA mutations assessed in PALOMA-3 study [21]. Plasma samples were collected at baseline, Day 15, and at progression. A reduction in CDR15 (circulating DNA ratio, defined as the ratio of mutant copies/ml at Day 15 on treatment relative to baseline) to <1 in *PIK3CA* was associated with improvement in PFS seen with palbociclib and fulvestrant. Thus, serial measurements of ctDNA can help characterize genomic landscape, clonal evolution, response and predict for early recurrence. However, it is not clear whether treatment decisions can be made based on the appearance of resistance mutations or MAF changes in ctDNA and that "genomically adapted therapy" can improve outcome [22]. *We hypothesize that real-time monitoring of ctDNA for secondary ESR1 alterations can identify subclinical progression and early intervention with ESR1 mutation-targeted agents can improve survival in ER positive MBC.*

1.3. CDK inhibitors

Currently, CDK inhibitors e.g. palbociclib (Ibrance), ribociclib (Kisqali), and abemaciclib (verzenio) are approved for the first line treatment of HR positive advanced breast cancers in combination with aromatase inhibitors as well as in combination with fulvestrant with disease progression following prior endocrine therapy. Final approval of palbociclib is based on data from PALOMA-2, which randomized 666 postmenopausal women (2:1) to palbociclib plus letrozole or placebo plus letrozole [23]. The PFS was 24.8 months in the palbociclib plus letrozole arm and 14.5 months in the placebo plus letrozole arm (HR=0.576, 95% CI: 0.463, 0.718, p<0.0001). Palbociclib is also approved in combination with fulvestrant, based on PALOMA-3, which randomized (2:1) a total of 521 pre-and postmenopausal HR positive women with MBC, with disease progression on or after prior adjuvant or metastatic endocrine therapy, to either palbociclib plus fulvestrant or placebo plus fulvestrant until disease progression [24]. The median PFS was 9.5 versus 4.6 months for patients treated in the palbociclib plus fulvestrant and placebo plus fulvestrant arms, respectively. Approval of ribociclib was based on MONALEESA-2, a double blind, placebo-controlled, trial in post-menopausal women with HR-positive, advanced breast cancers who received no prior therapy [25]. A total of 668 patients were randomized to receive ribociclib plus letrozole (n=334) or placebo plus letrozole (n=334). The estimated median PFS had not been reached in the ribociclib-containing arm and was 14.7 months in the placebo-containing arm. Objective response rate (ORR) in patients with measurable disease was 52.7% (95% CI: 46.6, 58.9) in the ribociclib plus letrozole arm and 37.1% (95% CI: 31.1, 43.2) in the placebo plus letrozole arm. Abemaciclib was initially approved in combination with fulvestrant for HR positive MBC patients who had progressed on prior endocrine therapy. This was based on MONARCH-2, a double-blinded, placebo-controlled study that randomized 669 patients to receive fulvestrant with or without abemaciclib. Median PFS was 16.4 months in patients taking abemaciclib plus fulvestrant vs. 9.3 months with fulvestrant alone [26]. Abemaciclib is also approved in

combination with aromatase inhibitor as initial therapy for HR positive MBC patients, based on the findings from MONARCH 3, a randomized (2:1), double-blinded, placebo-controlled, study in HR positive postmenopausal women with MBC [27]. The estimated median PFS was 28.2 in the abemaciclib-containing arm and was 14.8 months in the placebo-containing arm. In combination with endocrine therapy, CDK inhibitors are well tolerated with neutropenia, infections, leukopenia, fatigue, nausea, stomatitis, anemia, alopecia, diarrhea, thrombocytopenia, rash, vomiting, and decreased appetite being the most common adverse reactions.

1.4. Fulvestrant

Fulvestrant (Faslodex), a selective estrogen receptor antagonist, was initially indicated for treatment of HR positive MBC in post-menopausal women with disease progression following prior anti-estrogen therapy. It was recently approved by FDA as monotherapy for expanded use in post-menopausal women with HR positive advanced breast cancer, who have gone through menopause and have not received previous endocrine therapy. The FDA approval is based on data from the Phase III FALCON trial [28]. The FALCON trial is a Phase III, randomized, double-blind, multicenter trial that compared the efficacy and tolerability of 500mg dose of fulvestrant with anastrozole, in postmenopausal women with HR+, locally-advanced or metastatic breast cancer who have not received prior endocrine therapy. The study showed a statistically-significant increase in median PFS compared to anastrozole (16.6 vs. 13.8 months; HR: 0.797; p=0.049). The FALCON trial was designed on the basis of the Phase II FIRST trial, which demonstrated a median overall survival nearly six months longer with fulvestrant compared to anastrozole (54 vs 48 months; HR: 0.70; p=0.049)[29].

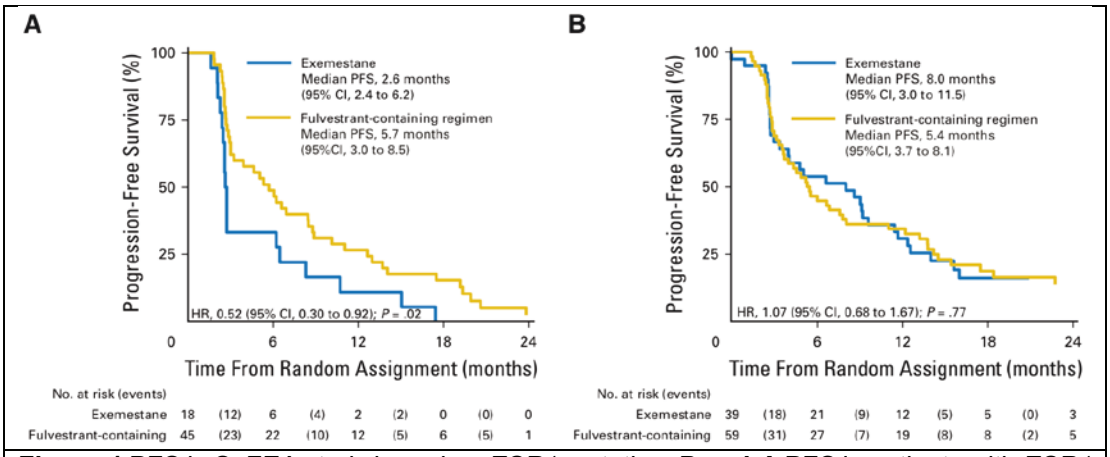


Figure-1 PFS in SoFEA study based on *ESR1* mutation. **Panel-A** PFS in patients with *ESR1* mutation treated with exemestane vs. fulvestrant containing regimen (A) Panel-B PFS in patients without *ESR1* mutation

Currently, the most effective therapy for patients harboring *ESR1* mutations is unknown. While preclinical studies have shown reduced sensitivity to fulvestrant at clinically achievable concentrations, no differences in progression-free survival (PFS) were noted in patients with plasma *ESR1* mutations treated with fulvestrant compared to wild-type (WT) in FERGI study [30]. FERGI study compared the activity of pictilisib, a pan-PI3K inhibitor, with fulvestrant in HR positive MBC patients with prior exposure to AI [30]. *ESR1* mutations were observed in about 37% of patients and was enriched in luminal A subtype, with similar PFS in *ESR1* mutant and wild type patients, suggesting that mutations are not associated with clinical resistance to fulvestrant. In PALOMA-3 study, an improvement in median PFS for combination of fulvestrant with palbociclib in MBC patients who had progressed on prior endocrine therapy (9.2 months vs 3.8 months for fulvestrant alone; P < 0.001) was observed. However, the use of fulvestrant with palbociclib was associated with similar improvement in progression free survival (PFS) in both wild-type and mutant *ESR1* suggesting that mutational status does not affect response to palbociclib [5]. In SoFEA trial, those with *ESR1* mutations had prolonged PFS with fulvestrant compared with exemestane ($P = 0.02$) while no PFS difference was observed in WT *ESR1* (**Figure-1**) [9]. Consequently, patients with *ESR1* mutations will be randomized to fulvestrant versus continuation of AI.

1.5. Guardant 360 Assay

ctDNA

Table-1 Guardant 360 ctDNA panel

Point Mutations (SNVs) (73 Genes)							Indels (23 Genes)		Amplifications (CNVs) (18 Genes)		Fusions (6 Genes)
AKT1	ALK	APC	AR	ARAF	ARID1A	ATM	ATM	APC	AR	BRAF	ALK
BRAF	BRCA1	BRCA2	CCND1	CCND2	CCNE1	CDH1	ARID1A	BRCA1	CCND1	CCND2	FGFR2
CDK4	CDK6	CDKN2A	CTNNB1	DDR2	EGFR	ERBB2	BRCA2	CDH1	CCNE1	CDK4	FGFR3
ESR1	EZH2	FBXW7	FGFR1	FGFR2	FGFR3	GATA3	CDKN2A	EGFR	CDK6	EGFR	NTRK1
GNA11	GNAQ	GNAS	HNF1A	HRAS	IDH1	IDH2	ERBB2	GATA3	ERBB2	FGFR1	RET
JAK2	JAK3	KIT	KRAS	MAP2K1	MAP2K2	MAPK1	KIT	MET	FGFR2	KIT	ROS1
MAPK3	MET	MLH1	MPL	MTOR	MYC	NF1	MLH1	MTOR	KRAS	MET	
NFE2L2	NOTCH1	NPM1	NRAS	NTRK1	NTRK3	PDGFRA	NF1	PDGFRA	MYC	PDGFRA	
PIK3CA	PTEN	PTPN11	RAF1	RB1	RET	RHEB	PTEN	RB1	PIK3CA	RAF1	
RHOA	RIT1	ROS1	SMAD4	SMO	STK11	TERT**	SMAD4	STK11			
TP53	TSC1	VHL					TP53	TSC1			
				** includes TERT promoter region			VHL				

analysis would be performed using the Guardant 360 assays (Guardant Health, Redwood City, CA). Guardant 360 is a CLIA-certified, CAP-accredited, New York State Department of Health approved digital sequencing technology for clinical cfDNA testing. The assay tests for SNVs (single nucleotide variations) in up to 73 genes, as well as indels, CNVs (copy number variations), and gene fusions in select genes. List of genes accessed by Guardant 360 is listed in **Table-1**. Patients with activating *ESR1* mutations identified on the Guardant 360 panel will be randomized into the study

1.6. Rationale

Nearly two-thirds of breast cancers overexpress estrogen receptor (ER) and estrogen blockade is an integral part of treatment. Currently the use of AI in combination with a CDK 4/6 inhibitor is recommended for first line treatment of ER positive MBC. Mutations in the ligand-binding domain of ESR1 have been described in MBC patients exposed to estrogen deprivation with AI. These mutations have been demonstrated to mediate resistance to AI therapy limiting their clinical activity and portend a shorter survival. Presently, the most effective therapy for patients harboring ESR1 mutations is unknown. Pre-clinical studies have suggested that fulvestrant, a SERD, is active in ESR1 mutant breast cancers. Analyses of circulating tumor DNA (ctDNA) offer a minimally invasive approach to monitor disease and treatment response. Currently, treatment decisions are based on clinical disease progression and thus at the time of high disease burden. Real time monitoring with ctDNA can identify patients who exhibit subclinical recurrence or progression. Early detection and intervention with alternate therapy to overcome resistance at minimal disease burden progression could have a larger impact than treating higher burden disease at clinical progression. However, it is not clear whether treatment decisions can be made based on the appearance of resistance mutations in ctDNA and that “genomically adapted therapy” can improve outcome. The present study hypothesizes that real-time monitoring of ctDNA for secondary ESR1 alterations can identify patients with subclinical progression and early intervention with ESR1 mutation-targeted agents at minimal disease burden progression. This strategy also precludes patients from being exposed to treatments known to be associated with resistance and unlikely to offer clinical benefit.

2. TRIAL OBJECTIVES AND ENDPOINTS

2.1. Primary Objectives

- To assess progression free survival (PFS) with transition to fulvestrant compared with continuing AI therapy in patients with emergence of *ESR1* mutations in plasma

2.2. Primary Endpoints

- Progression free survival as assessed by Kaplan-Meier analysis

2.3. Secondary Objectives

- To assess ctDNA *ESR1* mutant allele fraction (MAF) and kinetics with fulvestrant compared with AI
- To assess the prevalence of *ESR1* mutations in patients with secondary resistance to endocrine therapy
- To correlate ctDNA with CA 15-3 tumor marker changes
- To assess overall survival (OS) with transition to fulvestrant compared with continuing AI therapy in patients with emergence of *ESR1* mutations
- To assess PFS and time to next treatment (TTNT) on next line of therapy after progression on fulvestrant vs. AI in combination with CDKI

2.4. Secondary Endpoints

- MAF
- CA15-3
- OS
- PFS
- TTNT

2.5. Exploratory Objective

- Characterize other co-existing actionable genomic alterations of interest in relation to *ESR1* and clinical outcomes
- To determine frequency of other actionable genomic alterations and frequency of enrollment on genotype-matched therapy

2.6. Exploratory Endpoints

- Concomitant genomic alterations will be explored in relation to clinical outcomes of interest, and in particular in relation to the primary endpoint of PFS

3. STUDY DESIGN AND SELECTION OF PATIENTS

While the estimates of *ESR1* mutations in advanced breast cancers have varied across studies, data from SoFEA and PALOMA3 show that *ESR1* mutations were typically associated with sensitivity to prior endocrine therapy and secondary resistance. Secondary or acquired resistance is defined as relapse while on adjuvant endocrine therapy after at least 2 years, or relapse within 12 months of completion of adjuvant endocrine therapy or relapse after 6 months of endocrine therapy for MBC [31]. SoFEA trial enrolled patients with no disease progression or relapse on non-steroidal AI for at least 12 months in the adjuvant setting or at least 6 months in the metastatic setting as first line treatment. In SoFEA trial, nearly 90% of *ESR1* mutations observed in MBC patients who had received AI for at least 12 months. While PALOMA3 study enrolled a more varied population, 93% of *ESR1* mutations were observed in patients who were deemed sensitive to prior endocrine therapy suggesting that *ESR1* mutations are seldom associated with primary resistance [9]. Consequently, to identify *ESR1* mutations, HR positive (ER >10%) MBC patients who are on AI with CDK4/6 inhibitor (palbociclib, ribociclib, or abemaciclib) as first line therapy for at least 12 months would be screened for ctDNA analysis. Patients with *ESR1* alterations in ctDNA would be enrolled and randomized.

It is anticipated that approximately 400 MBC patients who are on AI with CDK4/6 as first line therapy for at least 12 months will be screened for ctDNA analysis in order to identify approximately 124 eligible patients with *ESR1* alterations in ctDNA. For screening, approximately 20 mL of peripheral blood will be collected in Streck™ tubes (see **Table 1**). The results from the screening ctDNA tests will be reported to treating physicians. Patients must meet all inclusion and exclusion criteria prior to treatment. The written informed consent must be obtained from the patient prior to screening. The following criteria apply to all patients enrolled onto the study unless otherwise specified**.**

3.1. Inclusion Criteria

1. Age ≥ 18 years.
2. ECOG performance status of 0 or 1.
3. Adequate bone marrow function as shown by: ANC ≥0.5 x 10^9/L, Platelets ≥50 x 10^9/L, Hb ≥7 g/dL
4. Adequate liver function as shown by:

   Total serum bilirubin ≤2.0 mg/dL,

   ALT and AST ≤2.5x ULN (≤5x ULN in patients with liver metastases),

   Adequate renal function: serum creatinine ≤ 2.0 x ULN;
5. Activating *ESR1* mutation (e.g. D538G, Y537S/N, S463P) identified on ctDNA. Novel *ESR1* alterations allowed as per discretion of PI
6. On AI with CDK4/6 inhibitor (palbociclib, ribociclib, or abemaciclib) as first line therapy for MBC for at least 12 months without evidence of clinical progression
7. Patients with histologically confirmed HR positive (ER+ and/or PR+ (>10%)), MBC

Since patients are already on AI and CDK4/6 inhibitors at the time of enrollment into the study and fulvestrant is the interventional drug, treatment can be administered as per standard of care parameters and at the discretion of treating physician

3.2. Exclusion Criteria

1. Pregnant or lactating women.
2. Received prior therapy for MBC (except for AI use for up to 4 weeks prior to initiation of CDK4/6 inhibitor).
3. Prior therapy with fulvestrant in the metastatic setting
4. HER2 positive MBC (as defined by ASCO/CAP guidelines)
5. QTc interval >480msec, Brugada syndrome or known history of QTc prolongation or Torsade de Pointes
6. Psychiatric illness, which would limit informed consent.
7. Patients who have any severe and/or uncontrolled medical conditions such as:
    - unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction ≤6 months prior to enrollment, serious uncontrolled cardiac arrhythmia, or any other clinically significant cardiac disease
    - active (acute or chronic) or uncontrolled severe infection, liver disease such as cirrhosis, decompensated liver disease, and active and chronic hepatitis (i.e. quantifiable HBV-DNA and/or positive HbsAg, quantifiable HCV-RNA),
    - severe hepatic impairment (Child-Pugh C)
8. HIV-positive patients on combination antiretroviral therapy
9. Expected survival < 6 months
10. Any serious medical illness, other than that treated by this study, which would limit survival to less than 1 month
11. Patients with a history of non-compliance to medical regimens or who are considered potentially unreliable or will not be able to complete the entire study;
12. Patients who are currently part of or have participated in any clinical investigation with an investigational drug within 1 month prior to dosing;
13. Women of child-bearing potential (WOCBP), defined as all women physiologically capable of becoming pregnant, must use highly effective methods of contraception during the study and 8 weeks after the end of treatment. Highly effective contraception methods include combination of any two of the following:
    - Placement of an intrauterine device (IUD) or intrauterine system (IUS);
    - Barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/ vaginal suppository;
    - Total abstinence or;
    - Male/female sterilization.
14. Male patients whose sexual partner(s) are WOCBP who are not willing to use adequate contraception, during the study and for 8 weeks after the end of treatment

4. REGISTRATION

4.1. Patient identification/Enrollment

HR positive (ER >10%) MBC patients who are on AI with CDK4/6 inhibitor (palbociclib, ribociclib, or abemaciclib) as first line therapy for at least 12 months would be screened for ctDNA analysis. Patients with positive *ESR1* mutations identified on ctDNA would be randomized to fulvestrant versus continuation of AI that they started on.

Patients must meet all of the eligibility requirements listed in Section 3.

4.2. Instructions for patients who do not start assigned protocol treatment

If a patient does not receive any assigned protocol treatment, baseline and follow-up data will still be collected. Document the reason for not starting protocol treatment on one of the baseline forms. Also report the date and type of the first non-protocol treatment that the patient receives.

5. TREATMENT PLAN

5.1. Treatment

- **Fulvestrant** 500 mg should be administered intramuscularly (+/- 3 days) into the buttocks slowly (1 - 2 minutes per injection) as two 5 mL injections, one in each buttock, on days 1, 15 of Cycle 1 and then Day 1 of Cycles 2 and beyond (Cycle = 28 days).
- **Ribociclib** tablets should be taken orally in combination with NSAI (non-steroidal aromatase inhibitor) or fulvestrant. Starting dose – 600 mg orally taken once daily for 21 consecutive days followed by 7 days off treatment.
- **Palbociclib** capsules should be taken orally with food in combination with an NSAI or fulvestrant. Starting dose – 125 mg orally taken once daily for 21 consecutive days followed by 7 days off treatment.
- **Abemaciclib** tablets should be taken orally in combination with NSAI or fulvestrant. Starting dose – 150 mg twice daily
- **Letrozole** 2.5 mg or **anastrozole** 1 mg orally should be taken orally daily
- Pre/Peri-menopausal women treated with CDKI plus NSAI or fulvestrant therapy should be treated with luteinizing hormone-releasing hormone (LHRH) agonists according to current clinical practice standards.

  Since treatment involves standard of care agents, patients can pursue treatment outside of MD Anderson, under the supervision of the local oncologist. However, clinical documents to confirm administration should be sent for review. Additionally, clinical assessments to confirm response (e.g. staging scans) should be performed at MD Anderson.

5.2. Drug Monitoring and Dose Modifications

Administration of palbociclib, ribociclib, or abemaciclib should follow standard procedure as outlined in the package insert. Dose interruption, modification or discontinuation based on individual safety and tolerability can be carried out as per investigator's or treating physician's discretion.

Palbociclib

The palbociclib prescribing information recommends monitoring complete blood counts prior to starting therapy and at the beginning of each cycle, as well as on day 15 of the first 2 cycles.

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

Based on pharmacokinetic analysis, mild (CrCl 60-90 ml/min) or moderate renal dysfunction (CrCl 30-60 ml/min) had no effect on the exposure of palbociclib. The pharmacokinetics of palbociclib have not been studied in patients with moderate or severe renal dysfunction.

Most common adverse reactions (incidence ≥10%) with palbociclib were neutropenia, infections, leukopenia, fatigue, nausea, stomatitis, anemia, alopecia, diarrhea, thrombocytopenia, rash, vomiting, decreased appetite, asthenia, and pyrexia.

Table-2: Recommended Dose Modification for Palbociclib

Dose Level	Dose
Starting dose	125 mg/day
First dose reduction	100 mg/day
Second dose reduction	75 mg/day
Third dose reduction	75 mg/day*

*Two weeks on, two weeks off. If further dose reduction is required, discontinue palbociclib

Ribociclib

The ribociclib prescribing information recommends monitoring complete blood counts and liver function tests (LFTs) prior to starting therapy and at the beginning of each subsequent 4 cycles and as clinically indicated, as well as on day 15 of the first 2 cycles. Additionally, EKGs prior to initiating therapy, Day 14 of first cycle, and the beginning of second cycle are required. Electrolytes at the beginning of each cycle for 6 cycles are also recommended.

No dose adjustment is necessary in patients with mild hepatic impairment (Child-Pugh class A). The recommended starting dose is 400 mg once daily for patients with moderate (Child-Pugh class B) and severe hepatic impairment (Child-Pugh class C)

Ribociclib should be avoided in patients who are at risk of developing QTc prolongation (e.g. long QT syndrome, electrolyte abnormalities, unstable angina or arrhythmias)

Most common adverse reactions (incidence ≥ 20%) are neutropenia, nausea, fatigue, diarrhea, leukopenia, alopecia, vomiting, constipation, headache and back pain.

Table-3: Recommended Dose Modification for Ribociclib	
Dose Level	Dose
Starting dose	600 mg/day
First dose reduction	400 mg/day
Second dose reduction	200 mg/day*

*If further dose reduction below 200 mg/day is required, treatment should be discontinued

Abemaciclib

The abemaciclib prescribing information recommends monitoring complete blood counts and liver function tests (LFTs) prior to starting therapy, every 2 weeks for the first 2 months, monthly for the next 2 months, and as clinically indicated.

No dosage adjustments are necessary in patients with mild or moderate hepatic impairment (Child-Pugh A or B). The recommended starting dose for patients with severe hepatic impairment (Child Pugh-C) is 150 mg daily.

Abemaciclib can increase serum creatinine due to inhibition of renal tubular secretion transporters, without any effect on glomerular function. Typical elevations happen during the first cycle, but they remained stable through the treatment period and were reversible with discontinuation. No dosage adjustment is recommended for patients with mild or moderate renal impairment (CrCl ≥ 30-89 mL/min). The

pharmacokinetics of abemaciclib has in patients with severe renal impairment (CrCl <30 mL/min), dialysis, or end stage renal disease is unknown.

Most common adverse reactions (incidence ≥20%) were diarrhea, neutropenia, nausea, abdominal pain, infections, fatigue, anemia, leukopenia, decreased appetite, vomiting, headache, alopecia, and thrombocytopenia

Table-4: Recommended Dose Modification for Abemaciclib	
Dose Level	Dose
Starting dose	150 mg twice daily
First dose reduction	100 mg twice daily
Second dose reduction	50 mg twice daily

Fulvestrant

Fulvestrant is administered intramuscularly 500 mg on days 1, 15 of Cycle 1, and once monthly thereafter (Cycle = 28 days). For patients with moderate hepatic impairment, fulvestrant should be administered at 250 mg on days 1, 15 and once monthly thereafter. Moderate hepatic impairment is defined as Child-Pugh class B

The most common, clinically significant adverse reactions, occurring in ≥ 5% of patients receiving fulvestrant are, pain at the site of injection, nausea/vomiting, bone pain, arthralgia, headache, fatigue, hot flashes, anorexia, asthenia, musculoskeletal pain, cough, dyspnea, and constipation. Increase in hepatic enzymes (transaminases and alkaline phosphates) were observed in more than 15% of patients, and was independent of the dose.

For detailed information on dose modification for fulvestrant see Packaging Insert.

5.3. Concomitant Therapy

Patients must be instructed not to take any medications (over-the-counter or other products) during the protocol treatment period without prior consultation with the investigator. All medications (other than study drug) taken within 28 days of starting study treatment through the 30-day safety follow up visit should be reported on the CRF.

All supportive measures consistent with optimal patient care should be given throughout the study. Exceptions include the use of growth factors to treat anemia or thrombocytopenia as such use has been associated with decreased survival and increased thrombotic events in patients with breast cancer. The use of myeloid growth factors in the form of G-CSF should follow ASCO guidelines. G-CSF can be administered to treat CDK inhibitor-induced neutropenia as determined by the treating physician.

Dose Modifications for Use with Strong CYP3A Inhibitors

Concomitant use of strong CYP3A inhibitors should be avoided with **palbociclib**. If patients must the co-administered with a strong CYP3A inhibitor, dose of Palbociclib should be reduced to 75 mg daily. Avoid concurrent of palbociclib with strong CYP3A inducers. The dose of sensitive CYP3A4 substrates with narrow therapeutic indices may need to be reduced when given concurrently with palbociclib.

Concomitant use of strong CYP3A inhibitors should be avoided with **ribociclib**. If patients must the co-administered with a strong CYP3A inhibitor, dose of ribociclib should be reduced to 400 mg daily. Avoid

concomitant use of ribociclib with strong CYP3A inducers. The dose of sensitive CYP3A4 substrates with narrow therapeutic indices may need to be reduced when given concurrently with ribociclib.

Concomitant use of ketoconazole should be avoided with **abemaciclib**. If patients must the co-administered with a strong CYP3A inhibitor, dose of abemaciclib should be reduced to 100 mg twice daily. Avoid concomitant use of abemaciclib with strong CYP3A inducers.

Patients should avoid pomegranates or pomegranate juice, grapefruit, all of which are known to inhibit cytochrome CYP3A enzymes and may increase the exposure to palbociclib and ribociclib.

Avoid concomitant use of drugs known to prolong QT interval such as antiarrhythmics (including, but not limited to amiodarone, disopyramide, procainamide, quinidine and sotalol), and other drugs known to prolong the QT interval (including, but not limited to, chloroquine, halofantrine, clarithromycin, haloperidol, methadone, moxifloxacin, bepridil, pimozide and ondansetron.

There are no known drug-drug interactions with **fulvestrant**

Cytochrome P450 inhibitors/inducers/substrates

A list of drugs that are strong inducers or inhibitors of CYP3A4 and sensitive substrates of CYP3A4 with a narrow therapeutic window is provided here:

CYP enzymes	Strong inhibitor	Strong inducer	Sensitive substrate	Substrate with narrow therapeutic range
CYP3A4	Boceprevir, clarithromycin, conivaptan, grapefruit juice, indinavir, itraconazole, ketoconazole, lopinavir/ritonavir, mibefradil, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, telaprevir, telithromycin, voriconazole	Avasimibe, carbamazepine, phenytoin, rifampin, St. John's wort	Alfentanil, aprepitant, budesonide, buspirone, conivaptan, darifenacin, darunavir, dasatinib, dronedarone, eletriptan, eplerenone, everolimus, felodipine, indinavir, fluticasone, lopinavir, lovastatin, lurasidone, maraviroc, midazolam, nisoldipine, quetiapine, saquinavir, sildenafil, simvastatin, sirolimus, tolvaptan, tipranavir, triazolam, ticagrelor, vardenafil	Alfentanil, astemizole, cisapride, cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus, tacrolimus, terfenadine

5.4. Adverse Events

Information about common side effects are already known about the agents (palbociclib, ribociclib, abemaciclib, letrozole, anastrozole, fulvestrant).

An adverse event (AE) is the appearance or worsening of any undesirable sign, symptom, or medical condition occurring after starting the study drug even if the event is not considered to be related to study

drug. Medical conditions/diseases present before starting study drug are only considered adverse events if they worsen after starting study drug. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy.

Adverse events (including lab abnormalities that constitute AEs) should be described using a diagnosis whenever possible, rather than individual underlying signs and symptoms. When a clear diagnosis cannot be identified, each sign or symptom should be reported as a separate AE.

As far as possible, each adverse event should be evaluated to determine:
1. The severity grade (CTCAE Grade 1-4).
2. Its duration (Start and end dates or if continuing at the Safety Follow-up Visit).
3. Its relationship to the study treatment (Reasonable possibility that AE is related: No, Yes).
4. Action taken with respect to study or investigational treatment (none, dose adjusted, temporarily interrupted, permanently discontinued, hospitalized, unknown, not applicable).
5. Whether medication or therapy was given (no concomitant medication/non-drug therapy, concomitant medication/non-drug therapy).
6. Outcome (not recovered/not resolved, recovered/resolved, recovering/resolving, recovered/resolved with sequelae, fatal, unknown).
7. Whether it is serious, where a serious adverse event (SAE) is defined as in the Serious Adverse Events subsection.

All adverse events should be treated appropriately. Such treatment may include changes in study drug treatment including possible interruption or discontinuation, starting or stopping concomitant treatments, changes in the frequency or nature of assessments, hospitalization, or any other medically required intervention. Once an adverse event is detected, it should be followed until its resolution, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study drug, the interventions required to treat it, and the outcome.

The investigator (or physician designee) is responsible for verifying and providing source documentation for all adverse events and assigning the attribution for all adverse events for subjects enrolled.
Protocol #2018-0287 will be monitored by the MD Anderson External Data and Safety Monitoring Board (EDSMB).

Laboratory test abnormalities

Laboratory abnormalities that constitute an adverse event in their own right (are considered clinically significant, induce clinical signs or symptoms, require concomitant therapy or require changes in study treatment), should be recorded on the Adverse Events CRF. Whenever possible, a diagnosis, rather than a symptom should be provided (e.g. anemia instead of low hemoglobin). Laboratory abnormalities that meet the criteria for Adverse Events should be followed until they have returned to normal or an adequate explanation of the abnormality is found. Laboratory abnormalities, that do not meet the definition of an adverse event, should not be reported as adverse events. A Grade 3 or 4 event (severe) as per CTCAE does not automatically indicate a SAE unless it meets the definition of serious as defined below and/or as per investigator's discretion. Grade 3 or 4 neutropenia is an expected, on-target, AE with CDK4/6 inhibitors. Thus, dose modifications for neutropenia should follow specific prescribing information brochure guidelines.

Recommended Adverse Event Recording Guidelines

Attribution	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Unrelated	Phase I	Phase I	Phase I	Phase I	Phase I

			Phase II	Phase II Phase III	Phase II Phase III
Unlikely	Phase I	Phase I	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III
Possible	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III
Probable	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III
Definitive	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III

Pregnancy

Based on animal studies, palbociclib, ribociclib, or abemaciclib can cause fetal harm when administered to pregnant women. In animal reproduction studies, administration of palbociclib during organogenesis resulted in embryo-fetal toxicity at maternal exposures that were ≥4 times the human clinical exposure based on area under the curve (AUC). In animal reproduction studies, administration of ribociclib to pregnant rats and rabbits during organogenesis caused embryo-fetal toxicities at maternal exposures that were 0.6 and 1.5 times the human clinical exposure, respectively, based on area under the curve (AUC). In animal reproduction studies, administration of abemaciclib during organogenesis was teratogenic and caused decreased fetal weight at maternal exposures similar to human clinical exposure based on AUC at the maximum recommended human dose. Pregnant women should be advised of potential risk to fetus and should use effective contraception during and for at least 4 weeks after the last dose of palbociclib, ribociclib, or abemaciclib.

Lactation

There is no information regarding the presence of palbociclib, ribociclib, or abemaciclib in human milk, their effects on milk production or the breast fed infant. Because of the potential for serious adverse effects in breast fed infants with palbociclib, ribociclib, or abemaciclib, lactating women should not breast feed during treatment and for 3 weeks after the last dose.

Serious Adverse Event (SAE) Reporting

An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the investigator or the Western Institutional Review Board (WIRB), it results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of

the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).

- Important medical events as defined above, may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator.

- All life-threatening or fatal events, that are unexpected, and related to the study drug, must have a written report submitted within 24 hours (next working day) of knowledge of the event to the WIRB.
- All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the WIRB in accordance with the timeframes and procedures outlined in the WIRB reporting requirements using the WIRB "Promptly Reportable Information" form to report the following information to within 5 days:

1. New or increased risk
2. Protocol deviation that harmed a subject or placed subject at risk of harm
3. Protocol deviation made without prior IRB approval to eliminate an immediate hazard to a subject
4. Audit, inspection, or inquiry by a federal agency
5. Written reports of federal agencies (e.g., FDA Form 483)
6. Allegation of Noncompliance or Finding of Noncompliance
7. Breach of confidentiality
8. Unresolved subject complaint
9. Suspension or premature termination by the sponsor, investigator, or institution
10. Incarceration of a subject in a research study not approved to involve prisoners
11. Adverse events or IND safety reports that require a change to the protocol or consent
12. State medical board actions
13. Unanticipated adverse device effect
14. Information where the sponsor requires prompt reporting to the IRB Information not listed above does not require prompt reporting to WIRB

Information not listed above does not require prompt reporting to WIRB. Please note, consistent with AAHRPP's requirements in connection with its accreditation of IRBs, the individual and/or organization submitting research for review shall promptly communicate or provide, and where necessary cause each investigator to promptly communicate or provide, the following information relevant to the protection of human subjects to WIRB in a timely manner:

a. Upon request of the IRB, a copy of the written plan between sponsor or CRO and site that addresses whether expenses for medical care incurred by human subject research subjects who experience research related injury will be reimbursed, and if so, who is responsible in order to determine consistency with the language in the consent document.

b. Any site monitoring report that directly and materially affects subject safety or their willingness to continue participation. Such reports will be provided to the IRB within 5 days.

c. Reports from any data monitoring committee, data and safety monitoring board, or data and safety monitoring committee in accordance with the time frame specified in the research protocol.
d. Any findings from closed research when those findings materially affect the safety and medical care of past subjects. Findings will be reported for 2 years after the closure of the research.

- Serious adverse events will be captured from the time of the first protocol-specific intervention, until 30 days after the last dose of drug, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.

Reporting to FDA:

It is the responsibility of the PI and the research team to ensure serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines and Western Institutional Review Board policy.

The Guardant 360 Assay is a liquid- based biopsy test with clinically minimal risks.

Device Failure

It is the responsibility of the PI and the research team to ensure unanticipated adverse device effects are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines and Western Institutional Review Board policy.

5.5. Duration of therapy

Patients will receive protocol therapy unless:

- Patient withdraws consent.
- Patient has progression of disease as defined in section 7.0.
- Patient experiences unacceptable drug toxicity.

Duration of Follow-up

For this protocol, all patients, including those who discontinue protocol therapy early, will be followed until 1 month after discontinuation of therapy for metastatic disease. Date of last follow up clinic visit should be within 30 days of end of treatment (+/- 10 days).

6. SCHEDULED EVALUATIONS

6.1. Pre-treatment evaluation

Baseline laboratory assessments should be performed as per standard of care for palbociclib, ribociclib, abemaciclib, and fulvestrant. Schedule of clinical assessments is listed in **Appendix 1**

- History and physical examination.
- Radiologic evaluation within 4 weeks prior to Cycle 1.
- Patient must sign IRB-approved informed consent prior to any study-specific procedures unless such procedures are part of the standard of care.
- Screening ctDNA Blood draw: Guardant360 StreckTM cell preservation tubes (8-10 mL x 2 per blood draw).

6.2. Evaluation during study

Follow up laboratory assessments should be performed as per standard of care for palbociclib, ribociclib, abemaciclib, and fulvestrant

- Physical examination (including vital signs, weight, performance status)
- Patients will undergo restaging with complete imaging (CT CAP and bone scan) approximately every 3-4 cycles of therapy for the first 12 months and every 3-6 months thereafter until progression.
- Exploratory ctDNA blood draws (Guardant360 StreckTM cell preservation tubes (8-10 mL x 2 per blood draw) will performed at Cycle 2, Cycle 4 and Cycle 12. These will be banked at MDACC. ctDNA will be drawn on the same date as standard of care labs assessments.
- End of Treatment/Progression ctDNA Blood draw (Guardant360 StreckTM cell preservation tubes (8-10 mL x 2 per blood draw).

6.3. Pregnancy and assessments of fertility

Based on animal studies, CDK inhibitors can potentially cause fetal harm when administered to pregnant women. In animal reproduction studies, administration of CDK inhibitors during organogenesis resulted in embryo-fetal toxicity.

Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, must use highly effective contraception during the study and for at least 4 weeks after stopping treatment. Highly effective contraception is defined as either:

- Total abstinence: When this is in line with the preferred and usual lifestyle of the subject. [Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception].
- Sterilization: have had surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least six weeks before taking study treatment. In case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment.
- Male partner sterilization (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate).
- Use of a combination of the following

  a. Placement of an intrauterine device (IUD) or intrauterine system (IUS).

b. Barrier methods of contraception: Condom or Occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/vaginal suppository.

Male Contraception

- Sexually active males must use a condom during intercourse while taking the drug and for 4 weeks after stopping treatment and should not father a child in this period.
- A condom is required to be used also by vasectomized men in order to prevent delivery of the drug via seminal fluid.
- Female partners of male patients must also be advised to use one of the following contraception methods: Use of (1) oral, injected, implanted or other hormonal methods of contraception, or (2) intrauterine device (IUD) or intrauterine system (IUS), or (3) prior male/female sterilization.

7. MEASUREMENT OF EFFECT

7.1. RECIST Criteria in metastatic disease

Tumor response for patients with measurable lesions should be assessed using RECIST 1.1[32]. Patients with measurable lesions should be assessed using contrast-enhanced CT of the chest, abdomen and/or MRI scan of the chest, abdomen and pelvis approximately (+/- 7 days) every three cycles (cycle length is 28 days), from the date of first dose of fulvestrant until the 30-day follow-up visit. Although progression may be determined by the investigator based upon clinical deterioration, every effort should be made to document progression using radiographic methods. The basis for determination of progression per clinical deterioration should be documented.

Note: It is very important that the same method of radiologic assessment be used throughout the study and that the same lesions are followed.

Response Criteria

Changes in the largest diameter of the tumor lesions and the shortest diameter in the case of malignant lymph nodes should be used in RECIST assessment.

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

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

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

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

8. BIOMARKER AND CORRELATIVE STUDIES

ctDNA analysis

To identify *ESR1* alterations, patients will have mandatory ctDNA assessment prior to randomization. In addition, to assess mutant allele fraction/ somatic allele burden (SAB) plasma for ctDNA would be obtained at 4, 12 weeks, and at progression. Blood draw for ctDNA would be stored in Guardant360 StreckTM cell preservation tubes (8-10 mL x 2 per blood draw). Where possible, blood samples for ctDNA will be drawn from patients scheduled to have venipuncture for standard of care labs assessments for clinical care. Patients with activating *ESR1* mutations will be treated until radiological disease progression. To characterize candidate mechanisms of resistance, Guardant 360 ctDNA analyses will be performed at disease progression and compared with pre-treatment ctDNA analyses. Specifically, we will evaluate alterations in *TP53* and *PIK3CA* and correlate with PFS. We will also evaluate for acquired *RB* mutations as well as other emergent genomic alterations with exposure to CDK inhibitors. We will correlate ctDNA dynamics with changes in CA 15-3 tumor marker. We will also correlate ctDNA dynamics and velocity with responses seen on imaging.

Solid Tumor Genomic Assay 2018									
Hotspot Genes n = 87				Full-Length n = 48		Copy Number n = 47		Gene Fusions n = 51	
AKT1	FGFR1	MAP2K1	RAF1	ARID1A	NOTCH3	AKT1	IGF1R	AKT2	MYBL1
AKT2	FGFR2	MAP2K2	RET	ATM	PALB2	AKT2	KIT	ALK	NF1
AKT3	FGFR3	MAP2K4	RHEB	ATR	PIK3R1	AKT3	KRAS	AR	NOTCH1
ALK	FGFR4	MAPK1	RHOA	ATRX	PMS2	ALK	MDM2	AXL	NOTCH4
AR	FLT3	MAX	ROS1	BAP1	POLE	AR	MDM4	BRAF	NRG1
ARAF	FOXL2	MDM4	SF3B1	BRCA1	PTCH1	AXL	MET	BRCA1	NTRK1
AXL	GATA2	MED12	SMAD4	BRCA2	PTEN	BRAF	MYC	BRCA2	NTRK2
BRAF	GNA11	MET	SMO	CDK12	RAD50	CCND1	MYCL	CDKN2A	NTRK3
BTK	GNAQ	MTOR	SPOP	CDKN1B	RAD51	CCND2	MYCN	EGFR	NUTM1
CBL	GNAS	MYC	SRC	CDKN2A	RAD51B	CCND3	NTRK1	ERBB2	PDGFRA
CCND1	H3F3A	MYCN	STAT3	CDKN2B	RAD51C	CCNE1	NTRK2	ERBB4	PDGFRB
CDK4	HIST1H3B	MYD88	TERT	CHEK1	RAD51D	CDK2	NTRK3	ERG	PIK3CA
CDK6	HNF1A	NFE2L2	TOP1	CREBBP	RB1	CDK4	PDGFRA	ESR1	PPARG
CHEK2	HRAS	NRAS	U2AF1	FANCA	RNF43	CDK6	PDGFRB	ETV1	PRKACA
CSF1R	IDH1	NTRK1	XPO1	FANCD2	SETD2	CDKN2A	PIK3CA	ETV4	PRKACB
CTNNB1	IDH2	NTRK2		FANCI	SLX4	CDKN2B	PIK3CB	ETV5	PTEN
DDR2	JAK1	NTRK3		FBXW7	SMARCA4	EGFR	PPARG	FGFR1	RAD51B
EGFR	JAK2	PDGFRA		MLH1	SMARCB1	ERBB2	RICTOR	FGFR2	RAF1
ERBB2	JAK3	PDGFRB		MRE11A	STK11	ESR1	TERT	FGFR3	RB1
ERBB3	KDR	PIK3CA		MSH2	TP53	FGF19	TSC1	FGR	RELA
ERBB4	KIT	PIK3CB		MSH6	TSC1	FGF3	TSC2	FLT3	RET
ERCC2	KNSTRN	PPP2R1A		NBN	TSC2	FGFR1		JAK2	ROS1
ESR1	KRAS	PTPN11		NF1		FGFR2		KRAS	RSPO2
EZH2	MAGOH	RAC1		NF2		FGFR3		MDM4	RSPO3
				NOTCH1		FGFR4		MET	TERT
				NOTCH2		FLT3		MYB	

For patients with tumor sites amenable to biopsy, repeat tumor biopsies at the time of disease progression may be performed as standard of care and subjected to tumor genomic analysis by next generation sequencing (NGS). Tumor genomic analysis would be performed using Oncomine Solid Tumor Genomics Assay 2018 (STGA2018, V3) in the Molecular Diagnostics Laboratory at MDACC. Tissue based tumor genome sequencing is approved in standard care for patients with metastatic breast cancers at MDACC by the Molecular Testing Evaluation Committee (MTEC). Genomic alterations identified by tissue based

NGS will be correlated with genomic alterations identified by ctDNA analysis. Genomic alterations assessed by STGA2018 are listed above.

9. STATISTICAL CONSIDERATIONS

Primary/Secondary Objectives:

This is a randomized, open-label, Phase II, for HR positive advanced breast cancer patients who have been treated with AI and CDK as first line in the metastatic setting for at least 12 months and who have *ESR1* mutations identified through ctDNA assay.

Becky Slack Tidwell, MS, Department of Biostatistics at MD Anderson Cancer Center, will oversee the implementation of the statistical analysis. The primary endpoint is PFS, which is measured from the date of study randomization to the date of event (ie, death and/or disease progression) or the date of last follow-up if no event has occurred. Survival curves for PFS will be analyzed using the Kaplan-Meier method and survival difference across groups will be examined by log rank test. Patients alive and disease-free at the latest clinical evaluation will be censored for PFS at the date of that evaluation. We will estimate the treatment comparison with 95% confidence intervals and p-value using Cox proportional hazards regression and we will assess the proportional hazard assumption using graphs of rescaled Schoenfeld residuals and related tests. We would attempt to identify independent predictors of PFS using multivariable Cox PH regression analysis. Analysis for OS and PFS from the start of next line of therapy will be performed as described for PFS from the date of randomization. Randomization will be performed using Core. Stratification factors include recurrence free interval (≤ 3 years vs. > 3 years), prior adjuvant endocrine therapy (tamoxifen vs. aromatase inhibitor), and site of metastasis (bone vs. visceral)

To detect a change in median PFS from 5 months (for AI arm) to 9 months (with fulvestrant arm) would require about 124 patients (assuming 4-5 patients per month accrual, 5 months post-accrual follow-up, 5% two-sided alpha, 80% power, log rank testing). 84 PFS events are expected under the alternative hypothesis. Interim analysis will be performed when 42 PFS events are observed. Using O'Brien-Fleming stopping boundaries, we will stop for futility if the log rank test p-value > 0.72 and stop for success if it is < 0.004. Descriptive statistics and graphical analysis will be used to summarize patients' demographic and clinico-pathological characteristics, and efficacy outcomes. We will use mean (standard deviation) or median (range) to summarize continuous variables and frequency (percentages) for categorical variables. Graphical analysis, such as scatter plots with Lowess smoothers, will be used to assess the correlative structure of outcomes and compare MAF between fulvestrant and AI treated groups. The Pearson correlation, or its non-parametric analogue, the Spearman correlation, will be used to estimate the linear correlation among variables. Radiographic response rate (partial response + complete clinical response) will be estimated and reported with a 95% CI.

Control Median	Experimental Median	Power
5	6	15%
5	7	38%
5	8	63%
5	9	81%
5	10	91%
5	11	96%
5	12	98%

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Appendix 1 Schedule of clinical assessments

Evaluation	Screening	Cycle 1	Cycle 2	Cycle 4	Cycle 12	End of treatment/ Progression
Physical exam, Vital Signs	X[a]					
ECOG PS	X[a]					
Hematology and Chemistry	Z					
Coagulation (PT/PTT/INR)	Z					
Pregnancy Test (urine)*	X					
Liquid Biopsy **	X**		X***	X***	X***	X**
Concomitant medications	X[a]					
Fulvestrant [d] administration		X	X	X	X	
Imaging	X[b]			X	X	X
Tumor marker	X		X	X[c]	X	

* For women of child bearing age. Repeated at the discretion of treating physician
[Z]Labs and clinical monitoring should be performed as per standard of care for CDK4/6 with AI/fulvestrant use
** Guardant ctDNA assessments are collected at screening (for baseline *ESR1* assessment), at progression.
***Exploratory samples for ctDNA assessment will be collected at Cycle 2, Cycle 4 and Cycle 12 and will be banked at MDACC and processed at Guardant Health at the time of study conclusion.

[a] All medications (other than study drug) taken within 28 days of starting study treatment through the 30-day safety follow up visit should be reported on the CRF.
[b] Tumor imaging (CT/Bone scan) will be performed approximately every 3-4 cycles of therapy for the first 12 months and every 3-6 months thereafter until progression. Tumor imaging should be performed at MD Anderson.
[c] Tumor markers (CA 15-3) performed approximately every 3 cycles (12 weeks) until disease progression in coordination with routine labs and imaging
[d] Fulvestrant administration for patients randomized to the fulvestrant arm only (+/- 7 days). Second dose of fulvestrant to be administered at C1D15. Control patients would continue the treatment they were already on (CDK inhibitor in combination with aromatase inhibitors). Fulvestrant can be administered under the supervision of treating physician locally.