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Treatment of Metastatic Breast Cancer with Fulvestrant plus Palbociclib or Tamoxifen plus Palbociclib: A Randomized Pilot Trial with ESR1 mutation tested in Circulating Tumor DNA.

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UPMC CancerCenter, Beaver
UPMC CancerCenter, Jefferson
UPMC CancerCenter at UPMC East (Monroeville)
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UPMC CancerCenter at UPMC Passavant (HOA)
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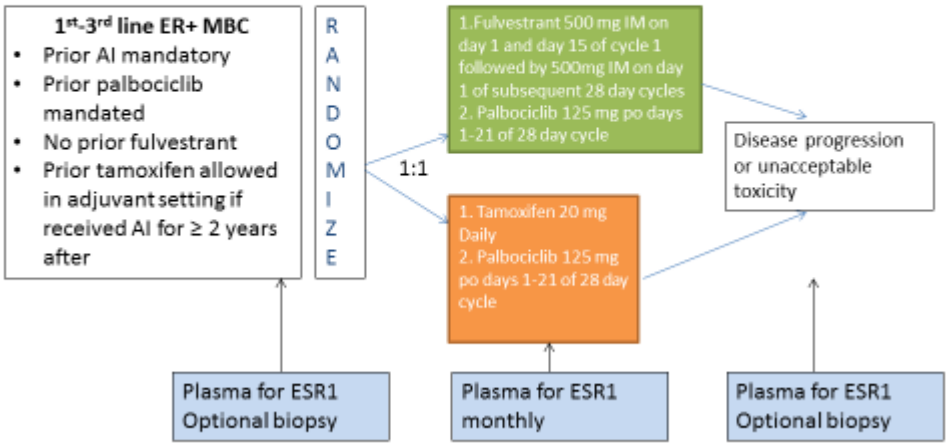

Patient Population (see section 4 for details)

- Second to third line in the metastatic setting
- Pre-menopausal (must receive chemical ovarian ablation) and post-menopausal
- All patients must have prior exposure to an AI in the adjuvant, neoadjuvant or metastatic setting
- Prior Palbociclib required
- Prior fulvestrant not allowed.
- Prior tamoxifen allowed in adjuvant setting only if followed by AI for ≥ 2years.
- Prior everolimus or other biologic agents are allowed.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations and special terms are used in this study Clinical Study Protocol.

TABLE 1. List of abbreviations

Abbreviation or special term	Explanation
AE	Adverse events
AI	Aromatase Inhibitor
CR	Complete response
CRS	Clinical research services
CTCAE	Common terminology criteria for adverse events
ctDNA	Circulating Tumor DNA
ddPCR	Droplet digital PCR
DMC	Data monitoring committee
DSMC	Data safety monitoring committee
eCFR	Electronic case report forms
ER+	Estrogen Receptor Positive
ESR1	Estrogen Receptor 1
ESR1-mt	Estrogen Receptor 1 mutant
ESR1-wt	Estrogen Receptor 1 wild type
FDA	Food and Drug Administration
GDP	Good clinical practice

Abbreviation or special term	Explanation
ICH	International clinical research
IRB	Institutional review board
ISS	Investigator sponsored study
LBD	Ligand binding-domain
MBC	Metastatic Breast Cancer
OS	Overall Survival
PD	Progressive disease
PFS	Progression Free Survival
PR	Partial response
PR	Progesterone receptor
RR	Response rate
SAE	Serious adverse events
SD	Stable disease
SRM	Study reference manual
TAM	Tamoxifen
TTP	Time to progression

1. INTRODUCTION

1.1 Background

A major challenge is to identify the drivers of progression from primary to metastatic breast cancer, and identify new targets for treatment. Mutations in the estrogen receptor (ESR1) are a potential resistance mechanism to endocrine therapy (1-9). In cell based-models, the presence of a mutation in ESR1 confers constitutive and ligand independent activity.(3) Although these mutations are extremely rare in previously untreated patients with early breast cancer, the development of these mutations over time after exposure to hormonal therapy with tamoxifen or AIs has been recently demonstrated to occur in as many as 14-54% of patients with estrogen receptor- positive (ER+) metastatic breast cancer (MBC).(7)

The mutations primarily occur in the ligand binding domain (LBD) of ESR-1 and there have been at least 6 mutations described. A recent study by Chandarlapathy identified additional (albeit very rare) ESR1 mutations (10) Patients whose tumors harbor *ESR1* mutations have poor overall survival. In a recent large meta-analysis including data from1540 patients, 28% had ESR mutations and had significantly worse PFS. The presence of ESR1 mutation, however, did not predict outcome on fulvestrant based therapy. (11) Even using a small cohort of patients, we reported shorter progression free survival (PFS) in patients with endocrine-resistant ER+ breast cancer receiving palbociclib who had *ESR1* mutations detected in cfDNA (12)

Together these observations support the tenable hypothesis that these mutations are clinically significant and the tumors that harbor them are strongly dependent upon ESR1 signaling.

Interestingly, in vitro, both tamoxifen and fulvestrant appear to have activity in the setting of ESR1 mutations; although different ESR1 mutations appear to have varying thresholds for effective dosing of both agents that define their level of partial resistance (3). Our group has recently performed detailed analysis of drug response in genome-edited T47D and MCF-7 cells, and these data were published Bahreini et al (13)Briefly, we introduced the most frequent ESR1 hotspot mutations D538G and Y537S, and observed not only ligand-independent activity of the mutant ER in growth and transcriptional activity assays, but also observed antiestrogen resistance: cells with mutant ER had higher IC50 for the SERMs 4OHT and raloxifene, and the SERDs fulvestrant and AZD9496 compared to WT (Figure 2A). We did observe differences between the mutants, with Y537S displaying increased resistance compared to D538G.

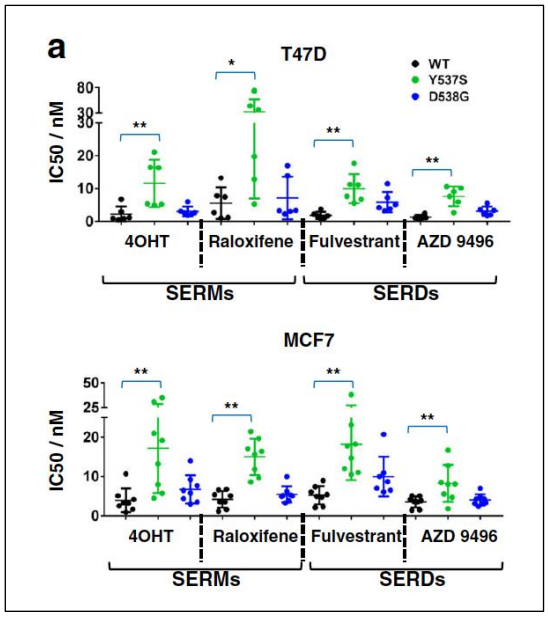

Figure 2: A) ESR1 mutant-cells display resistance against selective estrogen receptor modulators (SERMs) and selective estrogen receptor degraders (SERDs). Graphical (a) and tabular (b) presentation of half maximal inhibitory concentration (IC50) values that were determined in dose–response curves in wild-type (WT),

Y537S and D538G cells treated with 20 pM estradiol (E2) plus varying doses of 4OHT, raloxifene (Ral), fulvestrant (Ful), and AZD9496 in T47D and MCF7 cell lines. One-way analysis of variance was performed to compare the IC50 values of mutants to WT within each cell line and drug (*p < 0.05, **p < 0.01). (13).

Of note, our recent unpublished data shows metastatic properties of the D538G mutant receptor which might explain high MAF of this hotspot mutation despite decreased resistance compared to Y537S. We hypothesize that these novel gain-of-function activities are related to target genes specifically regulated by mutant ER (Figure 2B).

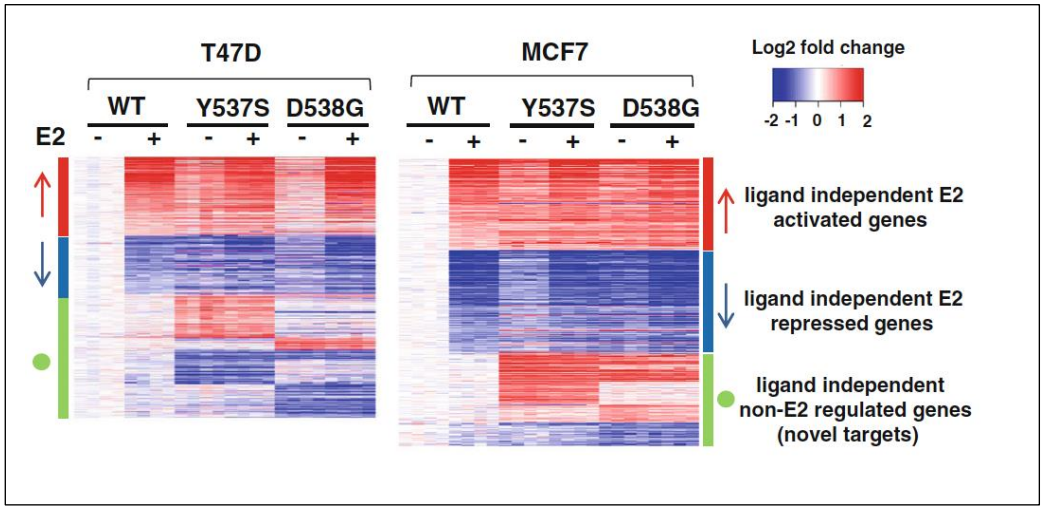

Figure 2B: Genome-wide transcriptomic analysis reveals regulation of ligand-independent estrogen receptor (ER) targets, and of novel target genes by ERα mutants in T47D and MC7 cells. T47D and MCF7 cell lines were hormone-deprived for 3 days, treated with vehicle (veh) or 1 nM of estradiol (E2) for 24 h, RNA was isolated and RNA sequencing analysis was performed. The heat map shows normalized log2 fold change (FC) of genes differentially regulated in mutants vs wild-type (WT) in the absence of ligand (FC >2, p value <0.005). The genes are sorted based on E2 regulation in WT (red arrow ligand-independent E2 activated genes, blue arrow ligand-independent E2 downregulated genes, green circle ligand-independent non-E2 regulated genes, i.e. "novel target genes"). (13).

As our studies indicate, the effect of different ESR1 mutations and their response to therapy are cell and context dependent. Thus it is important to determine the clinical relevance of these mutations and the efficacy of tamoxifen and fulvestrant in the setting of AI resistance especially since these two drugs possess distinct modes-of-action.

Specifically, as mutations in ESR1 appear to constitutively activate the receptor, the intrinsic ability of fulvestrant (in contrast to tamoxifen) to facilitate ER degradation may provide a mechanism to circumvent AI resistance with a more durable response. Our lab has recently published two publications on this important topic (17, 18).

Both fulvestrant and tamoxifen are standard hormonal therapies in patients with AI resistance. In a prospective setting, the efficacy and potential resistance of these agents in ESR1-mt tumors is unknown. There is some evidence that Fulvestrant treatment has efficacy in the tumors with ESR1 mutations, but this is less known for Tamoxifen. The prevalence of ESR1-

mt in the metastatic breast cancer population has been reported to be 15-50%. Some of these patients were heavily pre-treated endocrine- refractory patients. There is some but limited information on the prevalence of ESR1 mutations after AI failure and the incidence of ESR1 mutation as an effect of subsequent endocrine therapies. It is important to ascertain the prevalence of ESR1-mutations in patients earlier in their metastatic disease course, which is the time in which agents such as tamoxifen and fulvestrant are typically used. It is critical, therefore, to identify biomarkers in patient subsets that are predictive of rationale combinations of hormonal therapies as strategies to combat endocrine resistance from the outset.

Many patients now receive palbociclib with an AI in the first-line metastatic setting. ER-positive luminal breast cancer is particularly dependent on the interaction of cyclin Ds with the protein complexes CDK4 and CDK6 to drive cellular proliferation(18, 19). Together, they facilitate phosphorylation/inactivation of the Retinoblastoma tumor suppressor protein (RB), and this leads to progression through the G1 restriction point to the S phase of the cell cycle and, thus, cellular proliferation. Loss of RB is a potential mechanism of resistance to CDK4/6 inhibitors in vitro, but this needs to be validated in patients. Palbociclib (Ibrance, Pfizer) is an oral small molecule inhibitor of CDK4 and CDK6. Palbociclib received accelerated FDA approval based on the results of PALOMA-1, a randomized Phase 2 study comparing palbociclib plus letrozole versus letrozole alone in women with ER-positive HER-2-negative advanced breast cancer. PALOMA-1 showed significantly longer PFS with palbociclib plus letrozole (20.2 months) than with letrozole alone (10.2 months) (20)

The PALOMA-2 trial was designed as a confirmatory study and was a double-blind, randomized, Phase 3 2:1 comparison of palbociclib plus letrozole versus placebo plus letrozole. The median progression free survival was 24.8 months with the combination compared to 14.5 months in the placebo-letrozole group. The most common grade 3 or 4 adverse events were neutropenia, leukopenia, and fatigue (21).

The PALOMA-3 trial was a randomized, double-blind, placebo-controlled phase 3 trial comparing pelvis occluded in combination with fulvestrant compared with placebo plus fulvestrant in women with hormone receptor positive HER-2 negative metastatic breast cancer after progression on prior endocrine therapy. The median PFS on palbociclib plus fulvestrant was about 11 months, and it was not different in patients with greater than or equal to grade 3 neutropenia versus those who did not (22).

However, there is significant need for predictive biomarker development to guide therapy in patients who are resistant to AIs and/or have received prior palbociclib. Loss of RB is one potential biomarker of CDK4/6 resistance, but it is actually not commonly described in ER-positive breast cancer. Other potential biomarkers in include CCnE1, amplification of E2F, cyclin E levels, or loss of CDKN1A, that has been linked to tamoxifen resistance.

In addition, there is currently no data to support CDK4/6 beyond progression in patients with AI-resistant disease. It is unknown whether these agents can prevent emergence of resistance in patients formerly treated with an AI/CDK4/6 combination. It is also unknown whether they could circumvent the ligand-independent activation of ER signaling that occurs in the setting of an ESR1mutation. Limited ongoing studies seek to address this question. The BioPER trial (NCT013184090) is an open label non-controlled phase II trial assessing Palbociclib in combination with endocrine therapy of investigators choice after prior successful treatment with a Palbociclib containing regimen, looking for molecular profiles identifying patient who may benefit from continued Palbocicilib in this setting. This trial focuses on Rb status and related signaling as potential biomarkers.

A second study of Palbociclib after CDK and endocrine therapy (PACE) (NCT03147287) is examining Fulvestrant, Palbociclib and Avelumab for patients with metastatic disease. This trial is not estimated to be completed until 2024. A third ongoing trial (NCT02738866), phase II trial of Palbociclib and Fulvestrant in patients with HR+ MBC who have progressed on treatment with Palbociclib and an AI. There are currently no trials examining the combination of Palbciclib with Tamoxifen vs Palbociclib with Fulvestrant in patients previously exposed to a CDK4/6 inhibitor.

The focus of this study will be examination of ESR1 mutations as a biomarker. Both tamoxifen and fulvestrant are possible standard-of-care therapies in this clinical setting. We will assess the detection of ESR1 mutations in plasma over time on both therapies.. Additionally, since testing for ESR1 is not fully standardized and because it is a clinically relevant question, we will collect response data as measured by PFS for fulvestrant plus palbociclib vs tamoxifen plus palbociclibfor treatment of MBC, unselected by ESR1.

While previous studies have predominately evaluated for ESR1-mt in tumor tissues, use of novel technology to assess for ESR1-mt in plasma makes it clinically feasible to assess the variability of this marker over time in response to treatment. A PCR Sanger sequencing-based assay for detection of the five ESR1 mutations is available for solid tumor detection. A new next generation sequencing assay, MammaSeq^{SM}, which measures all reported ESR1 mutations, and mutations and copy number alterations in another 77 genes commonly mutated in breast cancer, is also under development in the UPMC Molecular Pathology laboratory and will be incorporated into this protocol. We have recently published on the generation and use of MammaSeq^{SM} (20) For detection of ESR1 mutations in plasma, a droplet digital PCR (ddPCR) assay is performed in the research laboratory of Drs. Adrian V. Lee PhD and Steffi Oesterreich. Using this assay, we have identified ESR1 mutations in 5/30 (16.7%) of patients with metastatic breast cancer, and when only considering those with an ER-positive primary breast cancer, we find ESR1 mutations in 5/20 (25%) of patients. The MammaSeq^{SM} capabilities would allow us to more comprehensively assess the initial presence and also the acquisition of mutations during treatment with tamoxifen plus palbociclib and fulvestrant plus palbociclib.

This may provide an additional means to discern differences between these two mechanistically distinct treatments and also enable us to target our analysis to more specifically determine the significance of ESR1 mutations per se as prognostic and predictive indicators.

1.2 Research hypothesis

1. In the 2nd to 3rd line of AI resistant WT ESR1 MBC, Fulvestrant plus palbociclib treatment will result in earlier detection of ESR1 mutations compared to treatment with tamoxifen plus palbociclib

2. In the 2nd to 3rd line of AI resistant mutant ESR1 MBC, Fulvestrant plus palbociclib will result in increased PFS compared with tamoxifen plus palbociclib

3. Change of allele frequency of ESR1 mutation is associated with PFS in all groups

1.2 Rationale for conducting this study

We propose a pilot clinical trial comparing the standard doses of fulvestrant plus palbociclib to tamoxifen plus palbociclib while evaluating subjects for the presence of ESR1 mutation. The goal is to obtain plasma, and tumor samples if available, that will look at the changes in time in the detection of ESR mutations while on non-AI therapy but with continued exposure to palbociclib. As both tamoxifen or faslodex would be standard next line options for patients progressing on AI, we would like to look at these two populations, This project will provide needed information regarding the prevalence of ESR1-mt in patients with ER+ MBC in the 2^{nd} -3^{rd} line setting when hormonal therapies are predominantly used. We will determine the presence of ESR1-mt by analyzing tumor biopsies and by making use of the novel technology of detection of ESR1-mt from circulating tumor DNA (ctDNA) by digital droplet PCR (ddPCR) in plasma. This technology allows the non-invasive detection and measurement of the level of circulating ESR1-mt over time and in response to treatment. Measuring ESR1 both in tissue and plasma will allow for determination of concordance between both sites, and may remove any bias that metastatic tumor heterogeneity could contribute. The tissues collected will also be assessed for other alterations in ESR1 such as amplifications or fusions, as well as assessment of ESR1 abnormalities on ER binding sites and whether this is altered after fulvestrant treatment. Our group has been able to demonstrate congruence between an ESR1 mutation found on liver biopsy and in plasma using ddPCR in a patient with ER+ MBC. (figure 4, unpublished and reference 23).

FIGURE 3. Detection of ESR1 mutation in liver metastasis and plasma

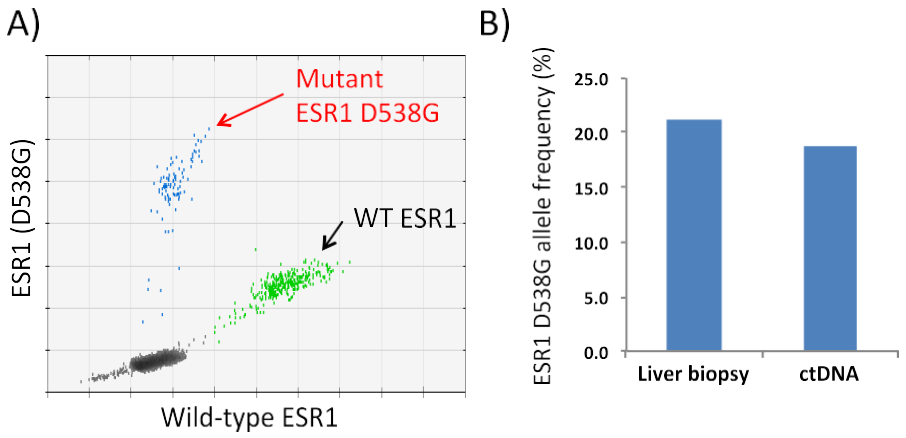

Figure 3: Detection of mutant ESR1D538G in a liver biopsy and circulating tumor (ct) DNA. A) Representative droplet digital PCR (ddPCR) using ~20ng of DNA isolated from a liver biopsy of metastatic breast cancer. B) Mutant ESR1D538G mutant allele frequency (mutant/wt alleles) in a liver biopsy of metastatic breast cancer and assoicated ctDNA. For ctDNA analysis 40ml of blood was collected in Streck tubes, plasma separated, DNA isolated and ddPCR performed using ~20ng of DNA.

This proposal will provide a prospective pilot study of the effect of the presence or absence of ESR1-mt on response to a commonly used therapy in the metastatic setting. This study will provide important data regarding a potentially very common and clinically relevant mutation that likely effects response to frequently used treatments. The results obtained can then be used as springboard to assess other potential therapies in this setting and to further study the biologic relevance of the mutation.

1.3 Benefit/risk and ethical assessment

This study will be conducted in accordance with the ethical principles that have their origin in the current Declaration of Helsinki and will be consistent with applicable US regulatory requirements and International Conference on Harmonization Good Clinical Practice (ICH GCP).

The study will be conducted in compliance with the protocol. The protocol and any amendments and the patient informed consent will receive Institutional Review Board (IRB) approval prior to initiation of the study.

Study personnel involved in conducting this trial will be qualified by education, training, and experience to perform their respective task(s). This trial will not use the services of investigators or study personnel where sanctions have been invoked or where there has been scientific misconduct or fraud (e.g., loss of medical licensure, debarment). Investigators are responsible for the conduct of the study at their study site.

2. STUDY OBJECTIVES

2.1 Primary objective

1. Assessment of longitudinal changes in allele frequency of ESR1 mutation in plasma in patients treated with Fulvestrant plus palbociclib compared to tamoxifen plus palbociclib

2. Assessment of genomic alterations (using MammaSeq) associated with wildtype or mutant ESR1

3. Assessment of the functionality of ESR1 in tumor biopsies collected prior to and following treatment with fulvestrant plus palbociclib, or tamoxifen plus palbociclib, as assessed by RNAseq

4. Safety and toxicity assessment

2.2 Secondary objectives

1. Collection of clinical outcome data in this limited patient population
    a. Progression free survival (PFS), Objective response rate (ORR) and Clinical benefit rate (defined as CR+PR+ stable disease at any tumor assessment) and clinical benefit rate at 6 months (CBR6) (defined as CR+PR+ stable disease for at least 6 months) in the entire cohort, and the ESR1-mt cohorts

2. Correlation between longitudinal changes in circulating levels of ESR1 mutations and clinical outcome
3. Assessment of concordance of ESR1 status in plasma and biopsy samples
4. Use of cfRNA to determine gene expression in AI resistant mutant ESR1 MBC

3. STUDY PLAN AND PROCEDURES

3.1 Overall study design and flow chart

Patients with ER+ breast cancer who had 1 to 3 prior lines of endocrine therapy and up to one line of chemotherapy for MBC, excluding fulvestrant and tamoxifen, will be randomized in a 1:1 ratio to receive fulvestrant 500mg IM Q28 days with one extra dose on D15 of the first cycle (as a loading dose) plus palbociclib 125mg/day PO on a 21 days on/7 days off schedule or tamoxifen 20mg PO daily plus palbociclib 125mg/day PO on a 21 days on/7 days off schedule. Patient accrual will be a goal of 40 evaluable at up to 4 sites. At randomization, plasma will

be obtained for evaluation of ESR1-mt and a biopsy will be obtained when possible. During treatment, all patients will undergo ct-DNA ESR1 mutation evaluation every 28 days. Treatment will continue until disease progression or unacceptable toxicity. At the time of disease progression, a second biopsy will be obtained when possible and the last blood drawn for evaluation of ct-DNA ESR1-mt will occur. All tissue obtained will also undergo molecular analysis to determine the activity of mutant ESR1.

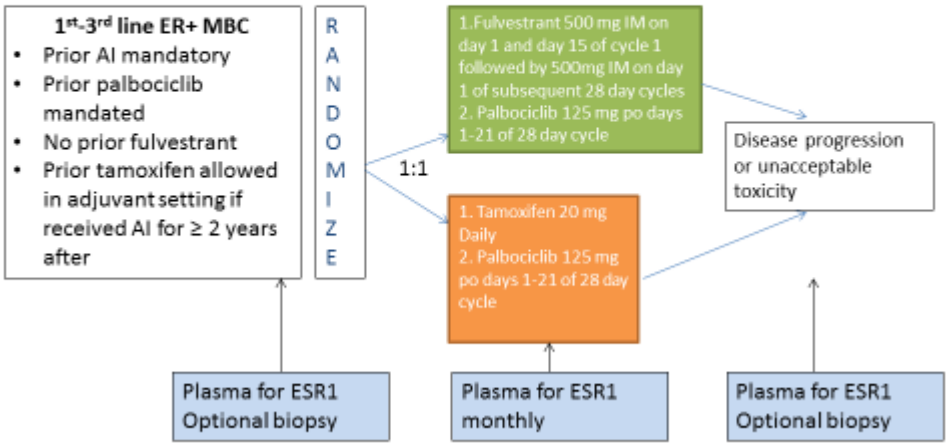

FIGURE 5. Study Schema

3.2 Rationale for study design, doses and control groups

Prior data supports the use of fulvestrant 500mg monthly with one extra dose 14 days after the first dose in metastatic breast cancer that is ER+. Two phase III trials comparing fulvestrant 250mg monthly to anastrozole published in 2002 and showing similar efficacy were the basis of approval by the FDA. (9) In 2004, Howell et al published the first trial comparing fulvestrant with tamoxifen in previously untreated MBC that was ER+ in postmenopausal woman. This study used the approved dose of fulvestrant in 2004 of 250mg IM monthly compared with tamoxifen 20mg PO daily and included 563 patients. Not all patients had known ER+ in the study and the primary outcome of time-to-progression (TTP) was analyzed both in the intention-to-treat (ITT) population and in the subgroup of known ER+ patients which corresponded to 78% of the initial cohort. Time to progression was similar in both analyses. Amongst all patients the TTP was 6.8 months for fulvestrant and 8.3 months for tamoxifen (p=0.08). Amongst ER+ patient the TTP was 8.2 months and 8.3 months, respectively (p=0.39). Both regimens were well tolerated and fulvestrant resulted in similar

incidence of nausea, injection site pain or inflammation and thromboembolic events (3.3% vs. 5.8%). Fulvestrant was associated with lower incidence of hot flashes (17.7% vs. 24.7%, p=0.05). (10)

The FIRST study evaluated fulvestrant 500mg monthly compared with anastrozole in the first line setting of ER+ MBC. This was a phase II study with 205 patients that used clinical benefit rate (CBR) as the primary endpoint. CBR was similar between both drugs, but time to progression, which was a secondary end point, was superior to fulvestrant 500mg monthly. (11) More recently, an update of the FIRST trial reported improved OS of fulvestrant 500mg monthly. (12) Based on this data, the FALCON study which is a phase III clinical trial comparing fulvestrant 500mg monthly with anastrozole and using PFS as the primary outcome was initiated. This study was recently reported and showed superiority of fulvestrant in the 1st line setting over anastrozole with median PFS in the fulvestrant arm of 16.6 months and 13.8 months with anastrozole (HR 0.797, 95% CT 0.637-0.999). (13) In 2014 the CONFIRM trial comparing fulvestrant 250 mg monthly with fulvestrant 500mg monthly reported improved OS for the higher dose. (14) Therefore, fulvestrant 500mg monthly is the current standard dose. Based on the data above and our own unpublished preclinical data showing a dose-response relationship of fulvestrant and inhibition of breast cancer cells, we hypothesized that fulvestrant 500mg IM monthly could result in longer PFS compared with tamoxifen. We also hypothesize that this effect could be more pronounced in endocrine resistant breast cancer cells through ESR1 mutation as shown above. Because of that, we propose a phase II trial comparing the PFS of fulvestrant 500mg IM monthly with that of tamoxifen in the 2nd to 3rd line setting of ER+ MBC and with the exploratory evaluation of ESR1 mutation from solid tumor biopsies and ctDNA. The randomized study will be open label (unblinded) because the therapies are different modalities (injection, oral) and blinding would therefore be an onerous burden to the patients. However, we emphasize that clinic visits and disease assessments are on the same schedule for both treatment arms.

4. SUBJECT SELECTION CRITERIA

4.1 Inclusion criteria

For inclusion in the study subjects should fulfill the following criteria:

1. Signed informed consent
2. Patients must have histologically or cytologically confirmed invasive breast cancer that is ER+ (>1% staining) with radiographical or clinical evidence of metastatic disease
    a. Measurable and/or non-measurable disease
3. Prior therapies:
    a. Patients must have previously received an aromatase inhibitor in the adjuvant, neo-adjuvant or metastatic setting.

   b. Patients must have previously received palbociclib in the adjuvant, neo-adjuvant or metastatic setting. If patient is currently taking palbociclib at time of screening for the trial they may continue taking palbociclib.

   c. The minimum duration of AI in the adjuvant setting is 2 years.
   d. There is no minimum duration of AI in the metastatic setting or neoadjuvant setting.
   e. Patients may have been previously treated with an mTOR inhibitor or other investigational agent in addition to an aromatase inhibitor.
   f. Prior treatment with tamoxifen is allowed in the adjuvant setting provided that it was followed by a minimum of 2 years of an AI.

4. Brain metastasis is allowed if previously treated, stable and off steroids for a minimum of 56 days
5. Age > 18 years
6. Male or female breast cancer is allowed
7. Patients may be pre- or post-menopausal; pre-menopausal patients must be on ovarian suppression and must be adequately suppressed on LHRH agonists with estradiol levels in the post-menopausal range
   a. Premenopausal patients cannot be pregnant and must agree to adequate birth control in addition to ovarian suppression. Agreement by the patient and/or partner to use highly effective, nonhormonal form of contraception or two effective forms of non-hormonal contraception. Contraception use should continue during the duration of study treatment and for at least 6 months after the last dose of study treatment.
8. ECOG performance status 0-2
9. Adequate bone marrow function as indicated by the following, within 14 days of enrollment:
   a. ANC $\geq$ 1500 cells/mm^3
   b. Platelets $\geq$ 100,000 cells/ mm^3
   c. Hemoglobin $\geq$ 9 g/dL
10. Adequate liver function, as indicated by the following, within 14 days of enrollment.
   a. Total bilirubin $\leq$1.5$\times$ upper limit of normal (ULN)
   b. AST $\leq$ 1.5 $\times$ ULN
   c. ALT $\leq$ 2.5 $\times$ ULN
   d. Alkaline phosphatase $\leq$ 2.5 $\times$ ULN with the following exception; ALP $\leq$ 5$\times$ ULN in patients with bone metastases.
11. Adequate hemostatic function as determined by PT, INR and aPTT < 1.5$\times$ ULN (unless on therapeutic coagulation, in which case the adequate level of anticoagulation will be determined by the investigator).
12. Adequate renal function, as indicated by creatinine $\leq$ 1.5 $\times$ ULN.

4.2 Exclusion criteria

Subjects should not enter the study if any of the following exclusion criteria are fulfilled

1. Prior therapy exclusions:

   a. Prior therapy with fulvestrant
   b. Prior therapy with tamoxifen in the metastatic setting
   c. More than 3 prior lines of endocrine therapy in the metastatic setting
   d. More than one prior line of chemotherapy in the metastatic setting
2. Washout of 2 weeks is required for aromatase inhibitors; washout of 4 weeks is required for, everolimus or other biological agents with the exception of Palbociclib.
3. Patients must not be receiving any other investigational agent.
4. Patients with symptomatic, untreated CNS metastases are not eligible.
5. Patients may not have significant concurrent illness, infection, pregnancy or lactation
6. Patients must not have a different active malignancy, except for skin basal cell carcinoma, skin squamous cell carcinoma and cervical intraepithelial neoplasia.

5. STUDY CONDUCT

5.1 Randomization Procedures

5.1.1 Regulatory Requirements

Before a site may enter patients, protocol-specific regulatory and other documents must be submitted to the Coordinating Center at the University of Pittsburgh Cancer Institute (UPCI), as noted in study materials. Detailed information regarding document submission and control is provided to each site in separate study materials.

Once all required documents are received, reviewed and approved by the Coordinating Center's designated representative(s), a patient may be enrolled.

5.1.2 Patient Randomization

A member of the study team will confirm eligibility criteria and complete the protocol - specific eligibility checklist. Registration must occur prior to the initiation of therapy. Any participant not registered to the protocol before treatment begins will be considered ineligible and registration will be denied.

The Coordinating Center (UPMC Hillman Cancer Center) will review the submitted documents in order to verify eligibility and consent. To complete the registration process, the Coordinating Center will:

1. Register the participant on the study with the UPMC Clinical Research Services Coordinating Center (CRSCC).

2. Upon confirmation of registration, the Coordinating Center will will provide the study specific participant case number, and assigned treatment arm to the participating institution.

Following registration, participants may begin protocol treatment and must begin treatment within 5 to 7 days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a participant does not receive protocol therapy 5 to 7 days following

registration, the participant's protocol status must be changed and the occurrence must be communicated to the UPMC CRSCC of participant status changes as soon as possible.

5.2 Treatments

5.2.1 Identity of investigational products (note commercial supply will be used for both drugs. Palbociclib will be provided Pfizer.)

Treatment	Dosage form and strength	Manufacturer
Fulvestrant	500mg IM Q28 days	AstraZeneca
Tamoxifen	20mg PO Qdaily	AstraZeneca
Palbociclib	125mg PO D1-21 Q28 days	Pfizer

5.2.1.1 Fulvestrant

Please refer to the package insert for complete prescribing and toxicity information.

1. Other Names
   ICI-182, 780; Faslodex
2. Classification
   Fulvestrant is an estrogen receptor down regulator. One of its main differences from tamoxifen and the other SERMs is its lack of agonistic activity. It represents a class of drug that binds, blocks and degrades the ER.
3. Mode of Action
   Estrogen Receptor Antagonist.
4. Storage and Stability
   Refrigerate, 2°-8°C (36°-46°F). To protect from light, store in the original carton until time of use.
5. Administration
   Fulvestrant 500mg should be administered intramuscularly into the buttocks slowly (1-2 minutes per injection) as two 5mL injections, one in each buttock.
6. Supply
   Fulvestrant is supplied as 50mg/ml injection in 5-mL syringes for intramuscular administration.
7. Incompatibilities
   No known drug-drug interactions.
8. Adverse Effects

   10% incidence reported:

   a. Endocrine & metabolic: Hot flushes (7% to 13%)

b. Hepatic: Alkaline phosphatase increased (>15%; grades 3/4: 1% to 2%), transaminases increased (>15%; grades 3/4: 1% to 2%)

c. Local: Injection site pain (12% to 14%)

d. Neuromuscular & skeletal: Joint disorders (14% to 19%)

1% to 10% incidence reported:

a. Cardiovascular: Ischemic disorder (1%)

b. Central nervous system: Fatigue (8%), headache (8%)

c. Gastrointestinal: Nausea (10%), anorexia (6%), vomiting (6%), constipation (5%), weight gain (≤1%)

d. Genitourinary: Urinary tract infection (2% to 4%)

e. Neuromuscular & skeletal: Bone pain (9%), arthralgia (8%), back pain (8%), extremity pain (7%), musculoskeletal pain (6%), weakness (6%)

f. Respiratory: Cough (5%), dyspnea (4%)

<1% incidence reported: (Limited to important or life-threatening; reported with 250 mg or 500 mg dose): Angioedema, hepatitis, hypersensitivity reactions, leukopenia, liver failure, osteoporosis, thrombosis, vaginal bleeding

5.2.1.2 Tamoxifen

Note: Please refer to package insert for complete prescribing and toxicity information.

1. Other Names
   Soltamox, Nolvadex

2. Classification
   Tamoxifen is a selective estrogen receptor modulator that has antagonistic and agonistic effect on the estrogen receptor, depending on the target tissue.

3. Mode of Action
   Selective Estrogen Receptor Modulator.

4. Storage and Stability
   Store at controlled room temperature, 20-25°C (68-77°F). Dispense in a well-closed, light-resistant container.

5. Administration
   20 mg orally daily.

6. Supply
   Tamoxifen is supplied in 20mg tablets in bottles containing 90 tablets.

7. Incompatibilities
   Please see the Tamoxifen Package Insert for more details on the known medication interactions.

8. Adverse Effects

10% incidence reported:

a. Cardiovascular: Vasodilation (41%), flushing (33%), hypertension (11%), peripheral edema (11%)
b. Central nervous system: Mood changes (12% to 18%), pain (3% to 16%), depression (2% to 12%)
c. Dermatologic: Skin changes (6% to 19%), rash (13%)
d. Endocrine & metabolic: Hot flashes (3% to 80%), fluid retention (32%), altered menses (13% to 25%), amenorrhea (16%)
e. Gastrointestinal: Nausea (5% to 26%), weight loss (23%), vomiting (12%)
f. Genitourinary: Vaginal discharge (13% to 55%), vaginal bleeding (2% to 23%)
g. Neuromuscular & skeletal: Weakness (18%), arthritis (14%), arthralgia (11%)
h. Respiratory: Pharyngitis (14%)
i. Miscellaneous: Lymphedema (11%)

1% to 10% incidence reported:

a. Cardiovascular: Chest pain (5%), venous thrombotic events (5%), edema (4%), cardiovascular ischemia (3%), angina (2%), deep venous thrombus (≤2%), MI (1%)
b. Central nervous system: Insomnia (9%), dizziness (8%), headache (8%), anxiety (6%), fatigue (4%)
c. Dermatologic: Alopecia (≤5%)
d. Endocrine & metabolic: Oligomenorrhea (9%), breast pain (6%), menstrual disorder (6%), breast neoplasm (5%), hypercholesterolemia (4%)
e. Gastrointestinal: Abdominal pain (9%), weight gain (9%), constipation (4% to 8%), diarrhea (7%), dyspepsia (6%), throat irritation (oral solution 5%), abdominal cramps (1%), anorexia (1%)
f. Genitourinary: Urinary tract infection (10%), leukorrhea (9%), vaginal hemorrhage (6%), vaginitis (5%), vulvovaginitis (5%), ovarian cyst (3%)
g. Hematologic: Thrombocytopenia (≤10%), anemia (5%)
h. Hepatic: AST increased (5%), serum bilirubin increased (2%)
i. Neuromuscular & skeletal: Back pain (10%), bone pain (6% to 10%), osteoporosis (7%), fracture (7%), arthrosis (5%), joint disorder (5%), myalgia (5%), paresthesia (5%), musculoskeletal pain (3%)
j. Ocular: Cataract (7%)
k. Renal: Serum creatinine increased (≤2%)
l. Respiratory: Cough (4% to 9%), dyspnea (8%), bronchitis (5%), sinusitis (5%)

Less than 1% or frequency not defined: Angioedema, bullous pemphigoid, cholestasis, corneal changes, endometrial cancer, endometrial hyperplasia, endometrial polyps, endometriosis, erythema multiforme, fatty liver, hepatic necrosis, hepatitis, hypercalcemia, hyperlipidemia, hypersensitivity reactions, hypertriglyceridemia, impotence (males), interstitial pneumonitis, loss of libido (males), pancreatitis, phlebitis, pruritus vulvae, pulmonary embolism, retinal vein thrombosis, retinopathy, second primary tumors, Stevens-Johnson syndrome, stroke; tumor pain and local disease flare (including increase in lesion size

and erythema) during treatment of metastatic breast cancer (generally resolves with continuation); uterine fibroids, vaginal dryness, visual color perception changes

5.2.1.2 Palbociclib

Note: Please refer to package insert for complete prescribing and toxicity information.

9. Other Names
10. Classification
CDK4/6 inhibitor.

11. Mode of Action
CDK4/6 inhibitor

12. Storage and Stability
Palbociclib capsules and blisters should be stored at controlled and monitored room temperature specified on the product labels. Further storage and stability conditions are stated in the palbociclib IB. Investigators and site staff are requested to check storage temperatures daily (i.e. manually or by using alarm systems to alert of any excursions) and ensure that thermometers are working correctly as required for proper storage of investigational products.

Deviations from the storage requirements, including any actions taken, must be documented and reported to AFT. Once a deviation is identified, palbociclib must be quarantined and not used until receipt of documentation of permission to use the investigational product.

Medication should be kept in a secured locked area at the study site in accordance with applicable regulatory requirements. Medication which has been returned by the patients should be stored separately from medication that needs to be dispensed.

13. Administration
125 mg orally daily, day 1-21 of a 28 day cycle.

Palbociclib should be taken orally, once per day with food around the same time, starting at the 125-mg dose. Treatment is continuous daily for 21 days, followed by 7 days off, to complete a 28-day cycle.

Expected toxicities and potential risks as well as allowable dose modifications are described in section 9. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant’s malignancy within the Treatment Phase.

Administration is performed on an outpatient, self-administration basis. On days when a patient is scheduled for a clinic visit (dispensing visit), the patient should take the scheduled

palbociclib dose once all visit assessments have been performed and are within acceptable range unless otherwise indicated.

Missed doses of palbociclib (meaning doses that are missed for one day) should not be made up. For example, if a dose is entirely missed for one day, dose should be skipped and NOT retaken the next day; patients should resume regular dosing as prescribed the following day. If a dose is vomited at any time after taking palbociclib, a replacement dose should NOT be taken. Patients who inadvertently take 1 extra dose during a day must skip the next day's dose and notify his or her treating physician. If a patient takes more than two doses of palbociclib in a day, the patient should bring this to the attention of his or her treating physician.

Patients should be instructed to record daily administration of the study drugs in a drug or medication diary.

14. Supply

Palbociclib will be manufactured by Pfizer and provided by the study Sponsor as capsules containing 75 mg, 100 mg, or 125 mg equivalents of palbociclib free base. Palbociclib will be supplied to sites in High Density Polyethylene (HDPE) bottles containing 75 mg, 100 mg, or 125 mg capsules and are labeled for clinical use.

15. Incompatibilities

Please see the Palbociclib Package Insert for more details on the known medication interactions.

16. Adverse Effects

Likely (greater than or equal to 50% chance that this will happen):
• Neutropenia. A condition in which the number of white bloods cells called neutrophils is abnormally low. This increases the risk of infection, which may be serious or life threatening. You will be monitored closely for this risk.
Frequent (between a 10 to 50% chance that this will happen):
• Fatigue. Participants experiencing fatigue while taking palbociclib should exercise caution when driving or operating machinery.
• Weakness
• Low number of red blood cells that can causes tiredness and shortness of breath. May require a blood transfusion. (Anemia)
• Diarrhea
• Nausea
• Low number of platelets, which may cause bleeding and bruising. Bleeding may be serious or life threatening and may require a blood transfusion.
• Decreased appetite
• Constipation
• Mouth blisters/sores

• Infection of the sinus or lung
• Vomiting
• Loss of touch or sensation of pins and needles or numbness on the skin

Occasional (Between a 1 to 10% chance that this will happen):
• Rash
• Bloating
• Temporary hair loss
• Swelling in extremities (hands and feet)
• Headache
• Nosebleed
• Muscle spasm
• Inflammation of the mucous membranes
• Fever
• Dry mouth
• Fever with dangerously low white blood cell count
• Abnormal taste

Rare (Less than a 1 % chance that this will happen):
• Abnormal electrical conduction within the heart which may lead to arrhythmias or irregular heartbeat

• A blood clot that causes a sudden blockage in a lung blood vessel, usually due to a blood clot that traveled to the lung from the leg. A pulmonary embolism is a serious condition that can cause: permanent damage to part of your lung from lack of blood flow to lung tissue; low oxygen levels in your blood; damage to other organs in your body from not getting enough oxygen. If a clot is large, or if there are many clots, a pulmonary embolism can cause death.

5.2.2 Doses and treatment regimens

Fulvestrant Arm

1. Loading dose: Patients will receive a loading dose of 500mg of fulvestrant administered as two 250 mg intramuscular (IM) injections, one into each buttock, on day 1 and day 15.
2. Maintenance Dose: Patients will receive fulvestrant 500mg IM administered as two 250mg intramuscular (IM) injections, one into each buttock, once every 4 weeks starting on day 29. Each cycle will consist of 28 days during which fulvestrant will be administered on day 1.
3. Palbociclib dose: Patients will receive 125mg PO days 1-21 of a 28 day cycle.

Tamoxifen Arm

1. Patients will receive tamoxifen 20mg PO daily
2. Palbociclib dose: Patients will receive 125mg PO days 1-21 of a 28 day cycle.

5.2.3 Dose adjustment/modifications

Typically both tamoxifen and fulvestrant are well tolerated standard agents. There are not any a priori dose modifications for toxicity, treatment should continue until progression occurs.

In unusual circumstances where fulvestrant or tamoxifen must be discontinued prior to disease progression due to intolerable fulvestrant-associated toxicity, the UPMC Hillman Cancer Center study PI should be contacted. Tamoxifen can be continued at investigator's discretion if thrombosis occurs and if adequately anti-coagulated.

Investigators can hold either agent for up to 3 weeks at their discretion (patient travel, clinic holiday, etc.). If the delivery of any study drug is delayed for more than 3 weeks due to toxicity, that drug should be permanently discontinued.

If an overdose occurs in the course of the study, then investigators or other site personnel inform appropriate AstraZeneca and Pfizer representatives **within one day**, i.e., immediately but no later than **the end of the next business day** of when the PI becomes aware of it. The designated AstraZeneca and/or Pfizer representative works with the investigator to ensure that all relevant information is provided to the AstraZeneca and Pfizer Patient Safety data entry sites.

Palbociclib dose modifications should be performed according to prescribing information.

5.3 Restrictions and concomitant therapies during the study

The following restrictions apply during the entire duration of the study:

1. No other investigational therapy is allowed during the study period. If an investigational therapy was used prior to study enrollment, a washout period of 28 days is required before enrollment.
2. No anticancer agents other than the study medications may be given to patients during the study period, with the following exceptions:
    1. Bisphosphonates, Zometa (zoledronic acid), or Xgeva (Denosumab)
    2. GnRH analog agents.
3. If any other anticancer agents are required for a patient then the patient must first be withdrawn from the study.
4. Concurrent radiation therapy is prohibited, however palliative radiotherapy may be considered on a case by case basis after discussion with the Study Chair.
5. Grapefruit and other citrus juices affect P450 and PgP activity. Concomitant use will be avoided. All CYP3A4 inducers and inhibitors should be avoided.
6. Medications that inhibit or induce CYP2D6, , CYP2C8/9 and CYP2D6 will be avoided and used at the discretion of the principal investigator or treating physician on a case by case basis. Please see appendix A for a list of these medications.
7. Anticoagulant medications can be used during the study period. The appropriate PT, INR and PTT levels will be determined at the discretion of the treating physician.

5.4 Treatment adherence

The PI will determine when lack of adherence to the study protocol should lead to removal from study. The patient removed for lack of adherence will still be included in the ITT analysis.

5.5 Discontinuation of treatment

Discontinuation of the fulvestrant plus Palbociclibor tamoxifen plus Palbociclib will occur in the case of unacceptable toxicity, disease progression, patient's own request, treating physician's discretion or if the study is closed.

5.6 Procedures for discontinuation of a subject from treatment

All patients who initiate protocol treatment will be included in the overall evaluation of response as part of the ITT analysis. All reasons for discontinuation of treatment should be documented clearly in the study record. In case of treatment discontinuation due to unacceptable toxicity as defined in section 6.4, the patients will still be included in the overall evaluation of response by ITT. If the study medication is discontinued at the patient’s own request, the reason for discontinuation from the study must be documented and the patient followed for outcomes by ITT analysis.

5.7 Training of study site personnel

The participating sites will provide experienced staff, and adequate equipment and facilities to support this clinical trial. The participating sites will also be responsible for research staff training in computer applications, human subject research, and HIPAA compliance, as well as the continuing education in these areas as required by local institutional standards.

5.8 Withdrawal from study

Withdrawal from study may occur at patient’s own request. The reason for discontinuation from the study must be documented. The patients will be included in the overall evaluation of response by ITT. The patient’s permission for active and passive follow-up will be sought, with response censored at the time of study withdrawal only if the patient withdraws consent for any follow-up.

6. COLLECTION OF STUDY VARIABLES

6.1 Recording of data

All information will be collected on study-specific case report forms by the study staff at each institution. The necessary forms will be provided to each site by the UPMC Hillman Cancer Center CRSCC. The

Coordinating Center staff will enter the data required by the protocol within 10 days into the Electronic Case Report Forms (eCRF). The Principal Investigator is responsible for assuring

that the data entered into eCRF is complete, accurate, and that entry and updates are performed in a timely manner.

6.2 Data collection at enrollment and follow-up

The completed forms will be forwarded vie email or fax to the Coordinating Center for central review and inclusion in the study dataset with relevant source documentation as outlined in the case report forms. The data submission schedule is as follows:

1. At the time of registration:
    a. Registration Forms
    b. Informed Consent Form (signed by the subject and approved investigator)
    c. Eligibility Checklist
    d. Source documents related to eligibility and randomization
    e. Research tissue samples with documentation, if baseline tumor biopsy is collected.

2. At the start of each new cycle:
    a. Adverse event forms
    b. Drug diary (both arms)
    c. Study blood along with documentation (sent on same day of draw)
    d. Pertinent medication list

3. Final Assessment (off-treatment):
    a. AE form
    b. Pertinent source documents
    c. Research tissue/blood samples with documentation

4. Follow-up (disease status, survival every 3 months)

All study data will be reviewed for completeness and accuracy by the Protocol Chair or his/her designee. The Principal Investigator (or his/her designee) at each respective institution is responsible for review, and ensuring the completeness and accuracy, of the data generated by his/her institution.

Peripheral blood collected during the study will be sent to UPMC Magee-Womens Research Institute for analysis of ESR1 mutation. Solid tumor biopsies will be sent to the Magee-Womens Hospital of UPMC Department of Pathology for analysis. When solid tumor biopsies are obtained, one core will be sent for MammoSeq testing, which will include ESR assessment as well as other genes described above. This test will be performed at the Division of Molecular Genomic Pathology of UPMC Magee-Womens Hospital. The other core will be used for the NanoString studies. A 3rd core, if obtained, will be banked as fresh frozen tissue to be used in the event that the two (2) other core biopsy samples do not contain sufficient usable tissue for either analysis of ESR1 mutation and/or MammoSeq testing .

6.3 Efficacy

Response and progression will be evaluated in this study using the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumours (RECIST) Committee (version 1.1). Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria. Patients will be evaluated for response after every 2 cycles of therapy. In the case of non-measurable disease (as defined in 6.3.2), progression of disease will be determined by the appearance of new metastatic lesions or clinical worsening secondary to the non-measurable metastatic site at the treating physician's discretion.

6.3.1 Measurable systemic disease

Tumor lesions: Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

- 10mm by CT scan (CT scan slice thickness no greater than 5 mm).
- 10mm calliper measurement by clinical exam (lesions which cannot be accurately measured with callipers should be recorded as non-measurable).
- 20mm by chest X-ray.

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

6.3.2 Non-measurable disease

All other lesions, including small lesions (longest diameter <10mm or pathological lymph nodes with ≥10 to <15mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

Special considerations regarding lesion measurability

Bone lesions, cystic lesions, and lesions previously treated with local therapy require particular comment:

Bone lesions:

- Bone scan, PET scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.
- Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by cross-sectional imaging techniques such as CT or MRI can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above.
- Blastic bone lesions are non-measurable.

Cystic lesions:

- Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.
- 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Lesions with prior local treatment:

- Tumor lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression after local treatment.

6.3.3 Specifications by methods of measurements

Measurement of lesions

All measurements should be recorded in metric notation, using callipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 28 days before the beginning of the treatment.

Method of assessment

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

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial and ≥10mm diameter as assessed using callipers (e.g., skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is suggested. When lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.

Chest X-ray: Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.

CT, MRI: CT is the best currently available and reproducible method to measure lesions selected for response assessment. Guidelines have defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5mm 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.

MRI is also acceptable in certain situations (e.g. for body scans).

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for

independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, laparoscopy: The utilisation of these techniques for objective tumor evaluation is not advised. However, they can be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response or surgical resection is an endpoint.

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

Cytology, histology: These techniques can be used to differentiate between PR and CR in rare cases if required by protocol (for example, residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain). When effusions are known to be a potential adverse effect of treatment (e.g. with certain taxane compounds or angiogenesis inhibitors), the cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment can be considered if the measurable tumor has met criteria for response or stable disease in order to differentiate between response (or stable disease) and progressive disease.

6.3.4 Response Assessment

Target Lesions

When more than one measurable lesion is present at baseline all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as **target lesions** and will be recorded and measured at baseline (this means in instances where patients have only one or two organ sites involved a maximum of two and four lesions respectively will be recorded).

Evaluation of Target Lesions

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

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

- Progressive Disease (PD): At least a 20% increase in the sum of 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 progression).

- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient

increase to qualify for PD, taking as reference the smallest sum of diameters while on study.

Non-target lesions

All other lesions (or sites of disease) including pathological lymph nodes should be identified as **non-target lesions** and should also be recorded at baseline. Measurements are not required and these lesions should be followed as 'present', 'absent', or in rare cases 'unequivocal progression'. In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression. In addition, it is possible to record multiple non-target lesions involving the same organ as a single item on the case record form (e.g. 'multiple enlarged pelvic lymph nodes' or 'multiple liver metastases').

When the patient only has non-measurable disease, the increase in overall disease burden should be comparable in magnitude to the increase that would be required to declare PD for measurable disease: i.e., an increase in tumor burden from "trace" to "large", an increase in nodal disease from "localized" to "widespread", or an increase sufficient to require a change in therapy.

Evaluation of Non-Target Lesions

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

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

- Progressive Disease (PD): Unequivocal progression of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression).

Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best overall response assignment will depend on the findings of both target and non-target disease and will also take into consideration the appearance of new lesions.

TABLE 2. Time to response definition

Target Lesions	Non-Target Lesions	New Lesions	Overall Response

CR	CR	No	CR
CR	Non CR/Non PD	No	PR
CR	Not evaluated	No	PR
PR	Non PD or not all evaluated	No	PR
SD	Non PD or not all evaluated	No	SD
Not all evaluated	Non PD	No	NE
PD	Any	Yes or no	PD
Any	PD	Yes or no	PD
Any	Any	Yes	PD

CR= complete response, PR= partial response, PD= progressive disease, SD= stable disease, NE= not evaluable.

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

Progression Free Survival

PFS is defined as the duration of time from time of randomization to time of progression or death, whichever occurs first. PFS for a subject without an event will be censored on the date of last tumor assessment. If an interval of 6 months passes without a tumor assessment, PFS will be censored at the time of the earlier tumor assessment, even if an event (progressive disease or death) is later observed.

6.3.5 Correlative Studies

We will determine the prevalence of ESR1 mutations in plasma and metastatic biopsies of patients with metastatic breast cancer. We have an ongoing program for Sanger sequencing based detection of ESR1-mut in tumor specimens in the Division of Molecular Genomic Pathology of UPMC Magee-Womens Hospital . Detection of ESR1-mut in ctDNA will be performed at the UPMC Pathology laboratories. Patients in the trial will have blood assessed serially for the presence or absence of ESR1-mt to determine the mutational prevalence in a 2^{nd} -3^{rd} line ER+ MBC population with prior tamoxifen/AI exposure. We will also be able to follow the ctDNA levels over time in response to treatment. Patients with disease amenable to biopsy will be offered the option and encouraged to have a metastatic site biopsy performed prior to study treatment and again at the time of progression. The hypothesis is that circulating levels of ESR-mut will be detectable in 25% of patients in this setting (consistent with our pilot study) and will decrease in response to fulvestrant. Correlation of plasma results and tumor results will help to determine concordance between these two measurements as well as to assess for potential heterogeneity between plasma and a specific metastatic tumor site. For instance, while ESR1-mut have been previously identified in a number of metastatic sites including lymph node and liver metastases, there has not been identification of these mutations in bone specimens. Whether this is an effect of the tissue processing or whether there is truly biologic relevance to this remains to be seen. We may find that in plasma the detection of ESR1-mut may be much higher than the prevalence seen in metastatic biopsies for that reason (i.e. bone only ER+MBC). We will also perform ddPCR and NGS in ctDNA and tumor biopsies in conjunction with AstraZeneca scientists, as an additional exploratory objective for ESR and other co-existent mutations of interest. .

These tumor biopsies will be assessed for the most commonly described ESR1 mutations using Sanger sequencing of the three ESR1 exons, which will assess for 23 different ESR1 mutations (including the 6 most commonly described: S463P, L536Q, Y537S, Y537C, Y537N, and D538G)). These biopsies will also undergo next generation sequencing for other mutations that may be associated with ESR1-mut and could affect response to fulvestrant. This will be done using the MammoSeq assay developed at the UPMC Hillman Cancer Center, which includes genes commonly mutated in breast cancer, common actionable mutations in non-breast cancers, rare but actionable mutations such as ErbB2, as well as copy number amplifications and deletions. This will allow for determination of other mutations, such as PIK3CA, that are frequently encountered in ER+ MBC and could either be passenger mutations with the ESR1-mut or driver mutations that will provide greater influence over response to therapy. (see Table 3) Assessment of these mutations may also help to identify subsequent therapies for patients. As there is not any prospective data that currently uses molecularly targeted therapies towards other cancer genes (with exception of ER and HER-2) to guide therapies as a standard of care, we do not feel this should influence subsequent enrollment onto this trial of a standardly used agent. The hypothesis is that while 25% of ER+ MBC patients will have the most common ESR1-mut present in tumor specimens; there may be additional patients who will have other ESR1 alterations that will affect ESR1 activity and response to treatment. We will ask for archived tumor samples as well so that we can determine if the ESR1 mutation was acquired as a result of previous endocrine therapy or present at the time of diagnosis.

Metastatic biopsies obtained in the clinical trial will also undergo molecular analysis to determine the activity of mutant ESR1. Although tumors harboring ESR1 mutations appear to be acquired as a result of estrogen deprivation therapy (1-6), and patients with these particular tumors have a much worse overall survival (7), relatively little is known, mechanistically, about the role these mutants have in metastatic disease progression and tropism. We hypothesize that ESR1 mutants confer a selective advantage due not only to their intrinsic constitutive activity in the relative absence of estrogen, but also to their propensity to preferentially transactivate specific target genes, in response to microenvironment cues, that are required for tumor cell survival, colonization, and metastatic outgrowth. Thus, we anticipate that ER target gene expression profiles obtained from primary tumors and their matched ESR1-expressing metastatic counterparts will display distinct qualitative and/or quantitative differences. Furthermore, we hypothesize that a subset of genes from these derived differential gene signatures will be shared in metastases from the same distant sites among different patients harboring one of several ESR1 point mutations or gene fusions that also confer constitutive activity. This convergent mutant ER gene signature could then provide mechanistic insights into ESR1 metastatic dependencies and thus serve as a prognostic indicator of metastatic progression (and tropism) and as a predictive marker of therapeutic response to HD fulvestrant and next generation mutant ER-directed SERDs, SERMs, and allosteric antagonists. Most importantly, this approach offers the opportunity to correlate specific target gene expression with fulvestrant dose and clinical outcome and thereby elucidate the molecular basis for ESR1 conferred partial resistance to fulvestrant. Identifying these mutant ER-dependent essential genes could then define particular targeted combination strategies to address this resistance.

Our laboratories have developed and implemented methods for determining gene expression profiles from formalin-fixed paraffin embedded (FFPE) solid tumor biopsies that will be collected. We will use NanoString nCounter technology, which enables a highly multiplexed direct profiling of individual RNA molecules without amplification and is ideally suited for degraded RNA from small biopsy material (requiring only 100ng of RNA). We previously used nCounter to measure expression of genes (n=158) in the five major breast cancer molecular tests (PAM50, Oncotype Dx, BCI, MammaPrint and EndoPredict) and in the 78 cases of primary breast cancer measured we found the platform to be robust and highly reproducible. For this proposal, we have developed a wild type ERa target gene expression profile (n=208 genes) which is robustly increased or decreased by estrogen stimulation of ERa. Initially, to help validate this approach for discerning differences in ER-target gene expression profiles between wildtype and mutant ER at different estrogen and fulvestrant concentrations, we used CRISPR/cas9 targeted genome editing to generate isogenic pairs of T47D-derived cell lines expressing wildtype ER and its mutant (D538G)-expressing counterpart. Since it is quite possible that mutant ER can have specific target genes distinct from those of wild type ER (and therefore not be represented in our current probe kit), we will also conduct a gene expression microarray analysis using the isogenic cell line pair to identify potential mutant ER-specific target genes and accordingly expand our NanoString-based probe kit. The effect of estrogen and fulvestrant on gene expression profiles will be measured in vitro to produce a pharmacodynamic profile of receptor activity

TABLE 3. MammoSeq gene list:

ABL1	CDK4*	FGFR4*	KIT	NOTCH1
AKT1	CDKN1B	FOXA1	KRAS	NRAS
AKT3*	CDKN2A	GATA3	MAP2K4	PAK1*
ALK	CTNNB1	GRB7*	MAP3K1	PDGFRA
AR*	DNAH14	HIST2H2BE	MAP3K4	PIK3CA*
ARID1A	EGFR*	HLA-A	MDM2*	PIK3R1
ATM	ERBB2*	HRAS	MDM4*	PTCH1
AURKA*	ERBB3*	IDH1	MET*	PTEN
AURKB*	ERBB4	IGF1R*	MLL3	RB1
BRAF	ESR1*	INPP4B*	MTOR	RET
BRCA1	EZH2	INSR*	MYC*	RPTOR
BRCA2	FGF19**	JAK2*	NCOA3*	RUNX1
CCND1*	FGFR1*	JAK3*	NCOR1	SMO
CCNE1*	FGFR2*	JUN	NCOR2	STK11
CDH1	FGFR3	KDR	NF1	TP53

6.4 Safety

6.4.1 Definition of adverse events

An adverse event is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (e.g., nausea, chest pain), signs (e.g., tachycardia, enlarged liver) or the abnormal results of an investigation (e.g., laboratory findings, electrocardiogram). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered. The term AE is used to include both serious and non-serious AEs. Definition of AE will be in accordance to

the CTCAE version 4.03. All grades of AE's will be captured, greater than grade 1. AE's will start to be captured on day 1 of study drug administration.

6.4.2 Definitions of serious adverse event (SAE)

A serious adverse event is an AE occurring during any study phase (i.e., run-in, treatment, washout, follow-up), that fulfils one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Results in a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above.

6.4.3 Recording of Adverse Events

General: All adverse events will be captured on the appropriate study-specific case report forms (CRFs).

Serious Adverse Events: All serious adverse events, regardless of causality to study drug, will be reported to the Principal Investigator and/or the Study Coordinator at each institution, and also to the Coordinating Center.

All serious adverse events must be reported to the Coordinating Center within 1 business day after the investigator becomes aware of the event. Events should be reported using a MedWatch form (3500) as available on the FDA website (http://www.fda.gov/Safety/MedWatch). Follow-up information must also be reported within 1 business day of receipt of the information by the investigator.

The Coordinating Center will disseminate information regarding serious adverse events to the participating sites within 5 days of review of the information by the Protocol Chair (or her designee in the event of extended absence) only in the case that the event(s) is believed to be related (i.e., possibly, probably, or definitely) to the study medication.

The following variables will be collected for each AE:

- AE (verbatim)
- The date when the AE started and stopped
- CTCAE grade
- Whether the AE is serious or not
- Investigator causality rating against the Investigational Product (yes or no)
- Action taken with regard to investigational product
- AE caused subject's withdrawal from study *(*yes or no*)*
- Outcome.

In addition, the following variables will be collected for SAEs:

- Date AE met criteria for serious AE
- Date Investigator became aware of serious AE
- AE is serious due to
- Date of hospitalization
- Date of discharge
- Probable cause of death
- Date of death
- Autopsy performed
- Causality assessment in relation to Study procedure(s)
- Causality assessment in relation to Other medication
- Causality assessment in relation to Additional Study Drug
- Description of AE.

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not a SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be a SAE.

Adverse Events based on signs and symptoms

When collecting AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

Adverse Events based on examinations and tests

If deterioration in a laboratory value/vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result/vital sign will be considered as additional information. Wherever possible the reporting investigator uses the clinical, rather than the laboratory term (e.g., anemia versus low hemoglobin value). In the absence of clinical signs or symptoms, clinically relevant deteriorations in non-mandated parameters should be reported as AE(s s).

Deterioration of a laboratory value, which is unequivocally due to disease progression, should not be reported as an AE/SAE.

Any new or aggravated clinically relevant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE.

Disease progression

Disease progression can be considered as a worsening of a subject’s condition attributable to the disease for which the investigational product is being studied. It may be an increase in the severity of the disease under study and/or increases in the symptoms of the disease. The development of new, or progression of disease, or death attributable to breast cancer should be considered as disease progression and not an AE. Events, which are unequivocally due to disease, should not be reported as an AE.

6.4.4 Reporting of serious adverse events

Investigators and other site personnel must inform the FDA, via a MedWatch form, of any serious or unexpected adverse events that occur in accordance with the reporting obligations of 21 CFR 312.32, and will concurrently forward all such reports to AstraZeneca and Pfizer. A copy of the MedWatch report must be faxed to AstraZeneca and Pfizer at the time the event is reported to the FDA. It is the responsibility of the investigator to compile all necessary information and ensure that the FDA receives a report according to the FDA reporting requirement timelines and to ensure that these reports are also submitted to AstraZeneca and Pfizer at the same time.

When reporting to AstraZeneca and Pfizer, a cover page should accompany the MedWatch form indicating the following:

- Investigator Sponsored Study (ISS)
- The investigator IND number assigned by the FDA
- The investigator's name and address
- The trial name/title and AstraZeneca ISS reference number

Investigative site must also indicate, either in the SAE report or the cover page, the causality of events in relation to all study medications and if the SAE is related to disease progression, as determined by the principal investigator.

Send SAE report and accompanying cover page to AstraZeneca by email to AE Mailbox Clinical Trial (TCS) <AEMailboxClinicalTrialTCS@astrazeneca.com> or by fax to 1-302-886-4114 (US Fax number). Email is the preferred method. Send SAE report and accompanying cover page to Pfizer by email to AE Mailbox Clinical Trial (TCS) <XXX> or by fax to 1-XXXXXX (US Fax number). Email is the preferred method.

Serious adverse events that do not require expedited reporting to the FDA need to be reported to AstraZeneca and Pfizer preferably using the MedDRA coding language for serious adverse events.

All SAEs have to be reported to AstraZeneca and Pfizer, whether or not considered causally related to the investigational product. All SAEs will be documented. The investigator is responsible for informing the IRB and/or the Regulatory Authority of the SAE as per local requirements.

All SAE's should be reported to AstraZeneca and Pfizer within 5 days.

Institutional Review Board

All adverse events (AEs) and serious adverse events (SAEs) will be reported to the IRB per current institutional standards. If an adverse event requires modification of the informed consent, these modifications will be provided to the IRB with the report of the adverse event. If an adverse event requires modification to the study protocol, these modifications will be provided to the IRB as soon as is possible.

6.4.5 Causality of Adverse Event by Study Compound

The causality of SAEs (their relationship to all study treatment/procedures) will be assessed by the investigator(s) and communicated to AstraZeneca.

- Definitely: An adverse event which has a timely relationship to the administration of the investigational drug/agent, follows a known pattern of response, for which no alternative cause is present.

- Probably: An adverse event, which has a timely relationship to the administration of the investigational drug/agent, follows a known pattern of response, but for which a potential alternative cause may be present.

- Possibly: An adverse event, which has a timely relationship to the administration of the investigational drug/agent, follows no known pattern of response, but a potential alternative cause does not exist.

- Unlikely: An adverse event which does not have a timely relationship to the administration of the investigational drug/agent, follows no known pattern of response, does not reappear or worsen after re-administration of the investigational drug/agent (if applicable), and for which there is evidence that it is related to a cause other than the investigational drug/agent.

- Unrelated: An adverse event, for which there is evidence that it is definitely related to a cause other than the investigational drug/agent. In general, there is no timely relationship to the administration of the investigational drug/agent, or if there is a timely relationship, the event does not follow a known pattern of response, and there is an alternative cause.

6.4.6 Time period for collection of adverse events

All serious adverse events must be reported to the Coordinating Center within 1 business day after the investigator becomes aware of the event.

Follow-up of unresolved adverse events

Follow-up information is sent to the same contact(s) to whom the original SAE Report Form was sent, using a new SAE Report Form stating that this is a follow-up to the previously reported SAE and giving the date of the original report. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, and whether the patient continued or withdrew from study participation.

7. BIOLOGICAL SAMPLING PROCEDURES

7.1 Solid Tumor Biopsy Samples

Patients who opt to undergo the optional biopsy and have tumors amenable to safely consider research biopsy, as determined by interventional radiology, will undergo a biopsy after signing consent and before initiation of study drug.

Patients who opt for the 2nd optional biopsy at the end of study participation will undergo the 2nd biopsy upon progression of disease or discontinuation of study drug due to any other reason (e.g. unacceptable toxicity, discontinuation of study drug at physician discretion, discontinuation of study drug at patient request).

Patients may opt to have the optional biopsy at the beginning of the study only, at the end of the study only, or both. Therefore, patients who decided against the optional biopsy at the beginning of the study may still opt to have the 2nd biopsy at the end of the study only, and should be encouraged by the local PI to do so.

When patients opt to have the optional biopsy, there will be 2-3 core biopsies obtained. Two of these cores will be paraffin embedded. One core will be sent for MammoSeq testing, which will include ESR assessment as well as other genes described above.

The other core will be used for the NanoString studies. A 3rd core, if obtained, will be banked as fresh frozen to be used in the event that the 2 other core biopsy samples do not contain enough usable tissue.

The core for MammoSeq will be performed as specified in the Laboratory Manual.

Leftover Samples

Any leftover study blood and tissue samples will be stored at **Magee-Womens Hospital Tissue Bank** or **the Magee-Womens Research Institute.** Samples will be de-identified and stored indefinitely. At enrollment, patients will have the option to allow that these samples be used in future research, given that consent for such is signed.

The study coordinator will keep a tumor sample log that includes the study number, a specimen serial number, the patient's name, time point in therapy, and the date and time that the sample was drawn. The sample will be labeled with a serial number only. The laboratory technician will keep a log with the specimen number, conditions, processing and storage information.

Note: The correlative sample collection schedules outlined above are based on an ideal subject. The sample schedule should be followed as closely as is realistically possible; however, the schedule may be modified due to problems such as scheduling delays or conflicts (e.g., clinic closure, poor weather conditions, vacations, etc).

7.2 Blood Samples

Peripheral blood will be collected for 2 purposes: laboratory monitoring of adverse event, and analysis of ESR1 mutation presence and frequency. The volume of blood required for each collection is 10mls, and 40mls respectively. The blood sample for monitoring adverse events

will be sent to the center's laboratory. Blood specimens for determination of the presence of ESR1 mutation in circulating tumor DNA by ddPCR will be drawn into 4- 10ml collection tubes, and store at ambient temperature. They will be delivered to the laboratory as specified in the Laboratory Manual within 3 days. In the laboratory, ctDNA and genomic DNA will be isolated and stored at –20°C, as described in the Laboratory Manual. These samples will be analyzed for ESR ctDNA internally and for dd PCR and NGS for other genes of potential interest in collaboration with AstraZeneca.

The study coordinator will keep a blood sample collection log that includes the study number, a specimen serial number, the patient's name, time point in therapy, and the date and time that the sample was drawn. The sample will be labeled with a serial number only. The laboratory technician will keep a log with the specimen number, conditions, processing and storage information.

The correlative sample collection schedules outlined above are based on an ideal subject. The sample schedule should be followed as closely as is realistically possible; however, the schedule may be modified due to problems such as scheduling delays or conflicts such as clinic closure, poor weather conditions, vacations, etc.

7.3 Leftover Samples

Any leftover study blood and tissue samples will be stored at **Magee-Womens Hospital Tissue Bank** or **the Magee-Womens Research Institute.** Samples will be de-identified and stored indefinitely. At enrollment, patients will have the option to allow that these samples be used in future research given that consent for such is signed.

7.4 Handling, storage and destruction of biological samples

Peripheral blood obtained for study monitoring of side effects and for analysis of ctDNA will be sent to the center's laboratory as described in section 7.2 and the Laboratory Manual. Remaining samples after analysis will be stored under the supervision of Dr. Oesterreich if the patient has agreed and signed informed consent allowing tissue storage.

7.5 Withdrawal of informed consent for donated biological samples

If any subject withdraws consent for donated biological samples, no further biological samples will be obtained. Of note, the case will still be included in the intention-to-treat analysis given that the subject has received at least one dose of the study compound. The obtained tissue will be analyzed, unless the patient has requested during study consent that all samples are destroyed.

8. ETHICAL AND REGULATORY REQUIREMENTS

8.1 Ethical conduct of the study

This study must be carried out in compliance with the protocol and Good Clinical Practice, as described in:

1. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.

2. US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).

3. Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

The investigator agrees to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice that it conforms to.

8.2 Ethics and regulatory review

- Institutional Review Board: Information regarding study conduct and progress will be reported to the Institutional Review Board (IRB) per the current institutional standards of each participating center.

- Food and Drug Administration (FDA): This trial does not involve an Investigational New Drug (IND) application and no reporting is required with regards to the clinical trial outlined at this time.

8.3 Informed consent

An investigator will explain to each subject the nature of the study, its purpose, procedures involved, expected duration, potential risks and benefits. Each subject will be informed that participation in the study is voluntary and that she/he may withdraw from the study at any time, and that withdrawal of consent will not affect his/her subsequent medical treatment. This informed consent will be given by means of a standard written statement and will be submitted for IRB approval prior to use. No patient will enter the study before informed consent has been obtained. In accordance with the Health Information Portability and Accountability Act (HIPAA), the written informed consent document (or a separate document to be given in conjunction with the consent document) will include a subject authorization to release medical information to the study sponsor and supporting agencies and/or allow these bodies, a regulatory authority, or Institutional Review Board access to subjects' medical information that includes all hospital records relevant to the study, including subjects' medical history.

8.4 Changes to the protocol and informed consent form

Any change or addition to the protocol can only be made in a written protocol amendment that must be approved by AstraZeneca, Health Authorities where required, and the IRB/IEC/REB. Only amendments that are required for patient safety may be implemented prior to IRB/IEC/REB approval. Notwithstanding the need for approval of formal protocol amendments, the investigator is expected to take any immediate action required for the safety of any patient included in this study, even if this action represents a deviation from the

protocol. In such cases, AstraZeneca and IRB/IEC at the study site should be notified of this action in accordance with local IRB requirements.

8.5 Audits and inspections

Source data/documents must be available to inspections by AstraZeneca designee or regulatory health authorities.

9. STUDY MANAGEMENT

9.1 TABLE 4. Study timetable and end of study

Procedure	Screening[a]	Visit 1	Every 4 weeks	Every 8 weeks	Off Treatment	Follow Up
INFORMED CONSENT	X					
CLINICAL ASSESSMENTS						
Physical Exam	X	X	X[b]		X	X[c]
Medical History	X	X				X[c]
Vital Signs, Height, Weight	X	X[d]	X		X	(X)
ECOG Performance Status	X	X	X		X	
LABORATORY TESTS:						
Hematology (CBC with diff, platelets)	X	X[e]	X[f]		(X)	
Chemistries and liver function tests [g]	X	X[e]	X[f]		(X)	
PT, INR, PTT[q]	X					
Estradiol [h]	X					
Pregnancy Test [i]	X			X		
IMAGING:						
CT Chest/Abd/Pelvis[j]	X[j]			X	(X)	(X)
Brain MRI	X[k]				(X)	(X)
TREATMENT:						
Tamoxifen		X[L]				
Fulvestrant		X	X[L]			
Palbociclib		X[P]				

OTHER ASSESSMENTS:						
Concomitant Medication Review	X	X	X		X	
Symptom/Toxicity Assessment	X	X	X		X[m]	
Follow-up/Survival					X[n]	X[c]
CORRELATIVE STUDIES						
Blood collection for ESR1-mt analyzed by ct-DNA	X		X		X	
Archived tissue sample (from breast primary)	X					
Optional Solid Tumor Biopsy	X[o]				X[o]	

a) Screening assessments are required within 28 days of initiation of treatment unless otherwise noted.
b) Required ≤ 1 day prior to each specified treatment (exceptions may be made to extend this window, with approval by the Protocol Chair/designee).
c) To continue until patient death
d) Height at baseline only
e) Required ≤ 14 days prior to first day of treatment; baseline labs may be used if within window.
f) Required ≤ 1 day prior to each specified treatment (exceptions may be made to extend this window, with approval by the Protocol Chair/designee).
g) Chemistries to include measurement of sodium, potassium, chloride, CO2, BUN, creatinine, glucose, total bilirubin, calcium, total protein, albumin, AST, ALT, and alkaline phosphatase.
h) Estradiol if required to determine post-menopausal status can be performed any time prior to Visit 1 treatment
i) Serum Pregnancy test is required for women of childbearing potential only. Required ≤ 7 days prior to first treatment (i.e., day 1) unless otherwise noted. All outcomes of pregnancy should be reported to the PI and AstraZeneca.
j) PET/CT is allowed and the choice between using a CT or PET with CT will belong to the treating physician. If a PET is chosen, the CT is mandatory and must be of diagnostic quality.
k) Only if known CNS metastasis
L) Fulvestrant will be dosed as follows: 2x250 mg injections on days 1, 15, 29, then next in 4 weeks (day 57) and continuing every 4 weeks thereafter. Tamoxifen will be dosed as follows: Tamoxifen 20mg PO Daily.
m) All subjects must have a final toxicity assessment at least 30 days following the last dose of study drug
n) Subjects will be followed approximately every 3 months after completion of study treatment for disease status and survival until death through clinic visit, phone call or record review.

o) Optional tumor biopsy will be performed ≤ 30 days prior to first day of treatment and within 30 days of treatment discontinuation.
p) Palbociclib will be dosed 125mg PO QD days1-21 of 28 day cycle
q) PT, INR, PTT only needs to be repeated for optional biopsies if the patient on anticoagulants

(X) = OPTIONAL

Note: Additional tests may be performed at the discretion of the treating investigator as clinically indicated. The sample collection schedules outlined above are based on an ideal subject. The sample schedule should be followed as closely as is realistically possible; however, the schedule may be modified due to problems such as scheduling delays or conflicts (e.g., clinic closure, poor weather conditions, vacations, etc.).

9.2 Study Management

Principal Investigator

The Principal Investigator is responsible for performing the following tasks:

1. Coordinating, developing, submitting, and obtaining approval for the protocol as well as its subsequent amendments.
2. Assuring that all participating institutions are using the correct version of the protocol.
3. Taking responsibility for the overall conduct of the study at all participating institutions and for monitoring the progress of the study.
4. Reviewing and ensuring reporting of Serious Adverse Events (SAE).
5. Reviewing data from all sites.

Coordinating Center

The UPMC Hillman Cancer Center Coordinating Center is responsible for performing the following tasks:

1. Ensuring that IRB approval has been obtained at each participating site prior to the first patient registration at that site, and maintaining copies of IRB approvals from each site.
2. Managing central patient registration.
3. Collecting and compiling data from each site.
4. Establishing procedures for documentation, reporting, and submitting of AE's and SAE's to the Protocol Chair, and all applicable parties.
5. Facilitating audits by securing selected source documents and research records from participating sites for audit.

Participating Sites

We currently estimate participation of 3 sites. Participating sites are responsible for performing the following tasks:

1. Following the protocol as written, and the guidelines of Good Clinical Practice (GCP).
2. Submitting data to the Coordinating Center.
3. Registering all patients with the Coordinating Center by submitting patient registration form, signed informed consent, and all required documentation promptly
4. Providing sufficient, experienced clinical and administrative staff and adequate facilities and equipment to conduct a collaborative trial according to the protocol.
5. Maintaining regulatory binders on site and providing copies of all required documents to the Coordinating Center.
6. Collecting and submitting data within 10 days.

Staffing

UPMC Hillman Cancer Center Coordinating Site - Staffing will include a Clinical Research Manager, Clinical Research Coordinator and Research Associate. The participating sites will provide experienced staff, and adequate equipment and facilities to support this clinical trial. The participating sites will also be responsible for research staff training in computer

applications, human subject research, and HIPAA compliance, as well as the continuing education in these areas as required by local institutional standards.

Documentation

Each participating site is responsible for submitting copies of all relevant regulatory documentation to the Coordinating Center. The required documents include, but are not limited to the following: local IRB approvals (i.e., protocol, consent form, amendments, patient brochures and recruitment material, etc.), IRB membership rosters, summary of unanticipated problems or protocol deviations, and documentation of expertise of the investigators. The Coordinating Center will provide each participating site with a comprehensive list of the necessary documents. It is the responsibility of the participating sites to maintain copies of all documentation submitted to the Coordinating Center.

Confidentiality

All unpublished information that the Coordinating Center gives to the investigator shall be kept confidential and shall not be published or disclosed to a third party without the prior written consent of the Protocol Chair (or her designee).

Record Retention

Following closure of the study, each participating center will maintain a copy of all site study records in a safe and secure location. Investigator records should be maintained for a minimum of 7 years after completion or discontinuation of the study or for longer if required by applicable local regulations.

Additional Information

Each participating site is responsible for submitting additional information as requested by the Protocol Chair (or her designee). The Coordinating Center may terminate the study at a participating site in the event that these conditions are not followed.

10. DATA MANAGEMENT

Data will be scanned to Coordinating site. Site personnel will review and issue queries as needed. Electronic data queries stating the nature of the problem and requesting clarification will be created for discrepancies and missing values. Designated investigator site staff are required to respond promptly to queries and to make any necessary changes to the data. Concomitant treatments and prior medications entered into the database will be coded using the WHO Drug Reference List, which employs the Anatomical Therapeutic Chemical classification system. Medical history/current medical conditions and adverse events will be coded using the Medical dictionary for regulatory activities (MedDRA) terminology. Laboratory samples for hematology, biochemistry, liver function tests, urinalysis, and coagulation will be performed locally. The occurrence of any protocol violations will be determined. After these actions have been completed and the data has been verified to be complete and accurate, the database will be declared locked

11. EVALUATION AND CALCULATION OF VARIABLES

11.1 Calculation or derivation of efficacy variable(s)

The primary objectives of the study are descriptive assessments of ESR1 mutation detection in plasma. One of the secondary endpoints of the study is PFS. PFS is defined as the duration of time from time of randomization to time of progression or death, whichever occurs first. PFS for a subject without an event will be censored on the date of last tumor assessment. If an interval of 6 months passes without a tumor assessment, PFS will be censored at the time of the earlier tumor assessment, even if an event (progressive disease or death) is later observed.If a patient has not had an event, PFS will be censored at the date of the last adequate tumor evaluation. PFS will also be censored at the date of tumor evaluation when the next tumor evaluation (or death) is >6 months after the previous evaluation without an event. PFS will be assessed via local radiology assessment according to RECIST 1.1. Although central review may occur as part of site calibration or an audit, the local assessment will be considered as the basis for the primary efficacy analysis. In the absence of measurable disease at baseline, the following criteria (per RECIST 1.1) will be used to assess disease progression among the patients with nonmeasurable lytic or mixed (lytic + blastic) bone lesions: 1) The appearance of one or more new lesions or 2) Unequivocal progression of existing non-measurable lesions.

Additional secondary endpoints include response rate, clinical benefit rate, (defined as CR+PR+ SD at any tumor assessment), clinical benefit rate at 6 months (CBR6), in the entire cohort, and the ESR1-mut cohorts. Response rate will be reported from best overall response according to RECIST 1.1 and as described in section 6.3. The response rate will then be compared amongst the tamoxifen plus palbociclib and fulvestrant plus palbociclib arms in the entire cohort and in the subgroup of ESR1-mt patients.

ESR1 mutation will be analyzed both from tumor biopsies and peripheral blood circulating tumor DNA. These tumor biopsies and peripheral blood circulating tumor DNA will be assessed for the following most commonly described ESR1 mutations using Sanger sequencing of the three ESR1 exons which will assess for 23 different ESR1 mutations including the 6 most commonly described (S463P, L536Q, Y537S, Y537C, Y537N, and D538G). Next generation sequencing (NGS) and/or digital droplet PCR (ddPCR) of blood and tumor specimens will also be performed to look at co-existent mutations. It will then be reported as the frequency of occurrence of ESR1 mutation in the cohort of enrolled patients as well as in the cohort of patients treated with fulvestrant plus palbociclib and tamoxifen plus palbociclib.

11.2 Calculation or derivation of safety variable(s)

All adverse events will be captured on the appropriate study-specific case report forms (CRFs) and will be calculated as a frequency of occurrence according to the CTCAE v4.03. The frequency of adverse events will categorized in accordance to the grading system present in CTCAE v4.03, using the highest grade recorded for each AE for each patient. AE rates will be calculated from the tamoxifen plus palbociclib and fulvestrant plus palbociclib cohorts and compared using the mid-p correction to Fisher’s exact test.

12 . STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION

12.1 Statistical analysis plan

The primary analysis will be conducted at least 6 months after the last patient is enrolled. Cox proportional hazards regression will be used to estimate hazard ratios comparing PFS for tamoxifen plus palbociclib and fulvestrant plus palbociclib. Fulvestrant will be considered promising for the unselected population if the fitted hazard ratio is < 0.8, and for the ESR1-mut subpopulation if the hazard ratio is < 0.65.

Response rate will be compared between treatment arms using the mid-P correction to Fisher's exact test for univariate comparisons, and logistic regression for exploratory multivariable models. Cox proportional hazards regression will be used for analyses with overall survival as the endpoint.

Adverse events will be reported for all patients in the safety cohort defined above, summarized by treatment group. Adverse events and serious adverse events will be tabulated in order of prevalence, with the highest grade reported by each patient.

Concordance of ESR1 status in plasma and biopsy samples will be assessed primarily by agreement regarding presence/absence of mutations for tissue and blood samples collected no more than 30 days apart and without intervening therapy. Kappa statistics adjusted for clustering (both pre-therapy and post-therapy time points for some subjects) will describe basic agreement. Additionally, between-subject and within-subject variation in mutant allele frequency will be compared using linear mixed models and the concordance correlation coefficient. This approach (complementary analysis of categorical and continuous versions of

ESR1 mutation status) may also be applied to assessment of within-patient longitudinal change. The primary analysis for these correlative studies will examine presence and allele frequency of any ESR1 mutation. Secondary analyses will examine and compare tissue-blood concordance and longitudinal change in liquid biopsy measures for specific ESR1 mutations. Level and change in circulating levels of ESR1-mt will be assessed as predictors of PFS using Cox proportional hazards regression with time-varying covariates. All hypothesis tests will be two-sided.

12.2 Sample size justification

The study sample size is restricted by external constraints: a planned study with a PFS endpoint powered to direct future therapy choices failed to accrue, in part because of the ever-shifting treatment landscape as described above. A study with n=40, 20 each receiving fulvestrant plus palbociclib or tamoxifen plus palbociclib, will allow sufficient exploration of a variety of study aims, in order to prioritize and inform the aims of future studies. If a sufficient number of accrued patients have ESR1-mt disease, it may be feasible to evaluate the association between mutational burden and response to continued endocrine+palbociclib therapy. Alternatively, there may be a strong signal for evolution of mutational frequency as different for patients receiving fulvestrant+palbociclib versus tamoxifen+palbociclib. Similarly, there may be a signal suggesting that continued endocrine+palbociclib therapy is associated with clinical benefit only in patients with ESR1-mt tumors who receive fulvestrant+palbociclib

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APPENDIX A

List of medications that interact with CYP2D6, CYP3A4/5, CYP2C8/9 and CYP2D6 and should be avoided:

alfentanil
alprazolam
alprenolol
amitriptyline
amlodipine
amodiaquine2
amphetamine
aprepitant
aripiprazole
astemizole
atomoxetine
atorvastatin
boceprevir
bufuralol1
buspirone
cafergot
carbamazepine
carvedilol
celecoxib
cerivastatin
cerivastatin
chlorpheniramine
chlorpromazine
cilostazol
cisapride
clarithromycin
clomipramine
clonidine
cyclosporine
dapsone

desipramine
dexamethasone
dexfenfluramine
dextromethorphan1
dextromethorphan2
diclofenac1
diltiazem
docetaxel
domperidone
donepezil
duloxetine
encainide
eplerenone
estradiol
felodipine
fentanyl
finasteride
flecainide
fluoxetine
fluvastatin
fluvoxamine
gleevec
glibenclamide
glimepiride
glipizide
glyburide
haloperidol
hydrocortisone
ibuprofen
imipramine
indinavir
irbesartan
irinotecan
lercanidipine
lidocaine
lornoxicam
losartan
lovastatin
meloxicam
methadone
methoxyamphetamine
metoclopramide
mexiletine
midazolam1
minaprine
nateglinide
nebivolol
nelfinavir
nevirapine
nifedipine2
nisoldipine
nitrendipine
nortriptyline
ondansetron
oxycodone
paclitaxel
paroxetine
perhexiline
perphenazine
phenacetin
phenformin
pimozide
piroxicam
progesterone
promethazine
propafenone
propranolol
quetiapine
quinine
repaglinide
risperidone
ritonavir
romidepsin
rosiglitazone
salmeterol
saquinavir
sildenafil
simvastatin
sirolimus
S-metoprolol
sorafenib
sparteine
sunitinib
suprofen
tacrolimus (FK506)
telaprevir
telithromycin
terfenadine
thioridazine
timolol
tolbutamide
tolbutamide1
torisel
torsemide
tramadol
trazodone
triazolam2
valproic acid
vemurafenib
venlafaxine
verapamil
vincristine
zakirlukast
zaleplon
ziprasidone
zolpidem
zuclopenthixol