

PROTOCOL NUMBER: 16-1001

PROJECT TITLE: Phase II trial of fulvestrant plus enzalutamide in ER+/Her2- advanced breast cancer

VERSION DATE: 08/27/2019

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STATEMENT OF COMPLIANCE

This is an investigator-initiated study. The lead principal investigator (PI), **Anthony Elias, MD**, is conducting the study and acting as the sponsor. As the sponsor-investigator, both the legal/ethical obligations of a PI and those of a sponsor will be followed.

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

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

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

Sponsor-Lead Principal Investigator: Anthony Elias, MD
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Signature

Site Principal Investigator: _____
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Signed: _____ **Date:** _____
Signature

PARTICIPATING SITES

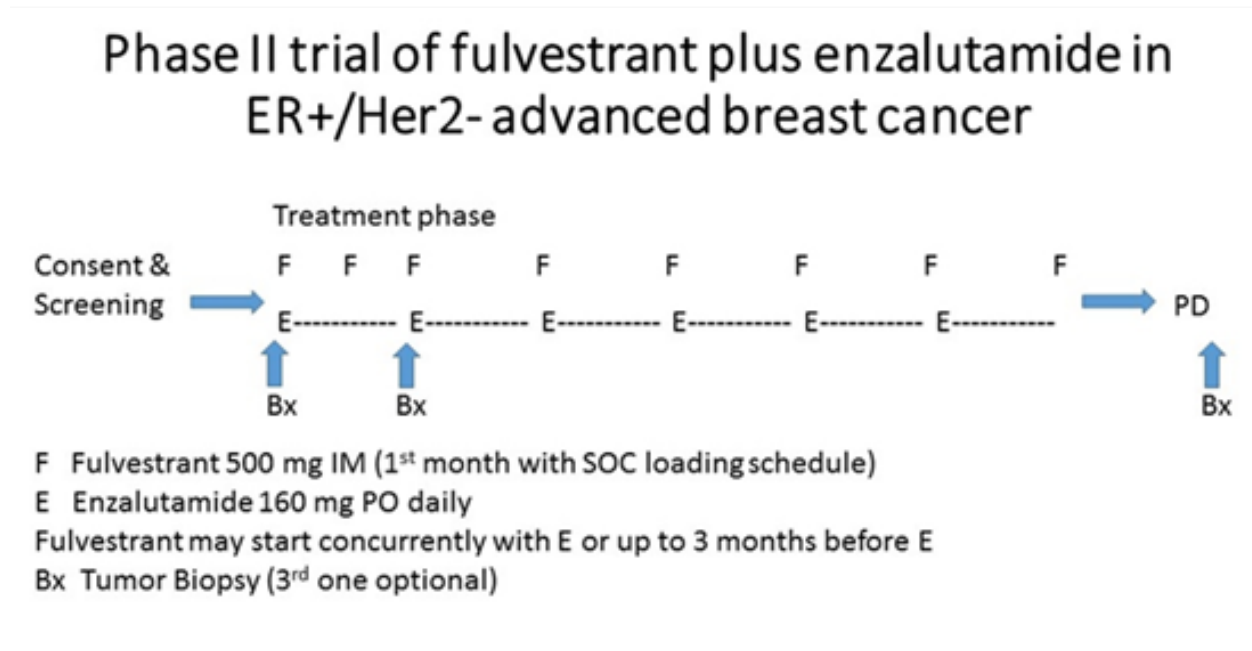
A complete and current listing of investigators, research personnel, research facilities and other study centers (if applicable) participating in this study will be maintained throughout the duration of this study on a **Protocol Contact List** form, incorporated herein by reference.

PROTOCOL/TRIAL SUMMARY

Protocol Title	Phase II trial of fulvestrant plus enzalutamide in ER+/Her2-advanced breast cancer
Protocol Number	16-1001
Trial Phase	II
Objectives	<p>Primary Objective: To determine the clinical benefit rate (complete and partial response plus stable disease for 24 weeks) of the combination of enzalutamide/fulvestrant.</p> <p>Secondary Objectives:</p> <ol style="list-style-type: none">1. To confirm the safety profile of the combination.2. Response rate (complete and partial response) at 24 weeks, Progression-free survival (PFS) and % of patients remaining free from progression at 12 and 24 weeks.3. To obtain serial biopsies of breast cancer pretreatment, during treatment and at time of tumor progression in order to determine the extent of AR expression and signaling in breast tissue, to evaluate the effect of enzalutamide on the tumor, and to evaluate the relationship of these effects on clinical outcomes.
Endpoints	<p>Primary Endpoint: <i>Clinical benefit rate at 24 weeks</i></p> <p>Secondary Endpoints: <i>Safety, PFS, PFS12, PFS24, ORR</i></p> <p>Exploratory Endpoint: <i>Tissue studies</i></p>
Subject Population	<p>Clinical Indication: ER+ Her2- breast cancer</p> <p>Gender: Female</p> <p>Age Range: 18-100 years</p>
Trial Type	Interventional
Type of control	Non-randomized
Description of Study Agents	<p>Fulvestrant: 500mg given IM on days 1, 15, and 28, and then every 4 weeks as per standard of care (SOC)</p> <p>Enzalutamide: 160mg given PO daily</p>
Trial Blinding	Open-label
Treatment Groups	Single arm

Number of trial subjects	24 evaluable; up to 45 may be consented to allow for screen failures
Estimated enrollment period	24 months
Duration of Participation	Until disease progression or unacceptable drug-related toxicity

SCHEMATIC OF STUDY DESIGN



LIST OF ABBREVIATIONS

ACRONYM	DESCRIPTION
AE	adverse event
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AR	androgen receptor
ASCO	American Society for Clinical Oncology
AST	aspartate aminotransferase
AUC	area under the curve
BC	breast cancer
CBC	complete blood count
CBR	clinical benefit rate
CFR	Code of Federal Regulations
cGCP	current good clinical practice

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CI	confidence interval
CLIA	Clinical Laboratory Improvement Amendments
CNS	central nervous system
CRF	case report form
CRPC	castration-resistant prostate cancer
CT	computed tomography
CTC	circulating tumor cell
CTCAE	Common Terminology Criteria for Adverse Events
CV	coefficient variation
DHT	dihydrotestosterone
DLT	dose-limiting toxicity
DSM	data and safety monitoring
DSMC	Data and Safety Monitoring Committee
eCRF	electronic case report form
ER	estrogen receptor
FDA	Food and Drug Administration
G(1/2)	grade of adverse event
IB	investigator's brochure
IHC	immunohistochemistry
IIT	investigator-initiated trial
IM	intramuscular
INR	international normalized ratio
IRB	institutional review board
LOI	letter of intent
MBC	metastatic breast cancer
mg	milligram
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
NCI	National Cancer Institute
NR	no response
OS	overall survival
PD	pharmacodynamics
PFS	progression-free survival
PI	principal investigator
PK	pharmacokinetics
PO	by mouth
PR	progesterone receptor
PS	performance status
PTT	partial thromboplastin time
R	response
RPMA	Reverse Phase Protein Microarray
SAE	serious adverse event
SOC	standard of care
TNBC	triple-negative breast cancer
UAP	unanticipated problem
UCCC	University of Colorado Cancer Center
USC	University of Southern California
ULN	upper limit of normal

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1. INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE:

1.1 Background Information

Breast cancer is considered a genetically heterogeneous and biologically diverse disease. We currently subdivide breast cancer by ER and Her2 status, in part because these markers represent important predictive biomarkers that guides treatment with discernible survival benefits. Endocrine therapies that target estrogen receptor (ER) signaling pathways play a critical role in the treatment of patients with ER+ disease, even in the advanced setting (1). The androgen receptor (AR) is expressed in most breast cancer specimens, though its functional role in initiating or driving malignancy is not yet well understood. In a study of 3093 breast cancers, AR expression (10% or more nuclear staining by immunohistochemistry [IHC]) was observed in 77% of invasive breast tumors and across all molecular phenotypes, but is expressed in up to 91% of ER+ BC (1-3).

In ER+ BC, AR expression is associated with resistance to anti-estrogen therapy. Tamoxifen-resistant breast tumors have a high ratio of AR-to-ER expression, while tumors that respond to tamoxifen express approximately equal amounts of AR and ER, as does adjacent uninvolved epithelium (4). Furthermore, a high ratio of percent cells positive for nuclear AR versus ER, a ratio of AR:ER ≥ 2.0 indicated an over four fold increased risk for failure while on tamoxifen (HR = 4.43) and had an independent effect on risk for failure above ER % staining alone. AR:ER ratio is also an independent predictor of disease-free survival (HR = 4.04, 95% CI: 1.68, 9.69; p = 0.002). One explanation for this is that AR signaling, which overlaps considerably with ER signaling, may take over from ER if ER signaling is blocked (5). This would lead to tumor cell survival and proliferation. In addition, the mechanism of action of an aromatase inhibitor is to block the conversion of androstenedione to estrogen, thereby increasing the concentrations of androgens that could, in turn, potentially stimulate the AR on tumor cells to enhance growth (6).

The anti-AR therapy enzalutamide blocks not only dihydrotestosterone (DHT)-mediated growth of BC cells, but also estradiol-mediated growth. In addition, in preclinical models of ER+/AR+ breast cancer enzalutamide is just as effective as tamoxifen in blocking estradiol-mediated growth *in vivo* (4). These findings suggest that AR influences breast tumor biology and is an independent predictor of response to anti-estrogen therapies, but perhaps even more importantly, that those patients who relapse on anti-ER therapies may benefit from anti-AR therapies, either up-front in combination with ER-targeted therapies, or following disease progression. New evidence is arising that nuclear-translocated AR serves as an important co-factor regulating DNA binding of activated ER, and that AR signaling may become important for tumor cell survival in the absence of estradiol (5).

In summary, although effective hormonal therapies are available for the treatment of ER+/PgR+ advanced breast cancer, advanced disease remains incurable and currently approved treatments have significant side effects. Taken together, nonclinical and clinical data suggest a potential role of AR signaling in the development of resistance to hormonal treatment. Improvements in the identification of biomarkers that predict benefit

from a given therapy are needed to ensure that patients receive the agents from which they are most likely to benefit and to minimize their exposure to toxic agents unlikely to provide benefit. The phase 2 study described herein is designed to address these needs.

1.2 Summary of Relevant Clinical Experience with Enzalutamide

Medivation and Astellas Pharma, Inc. are co-developing enzalutamide for the treatment of cancer. Enzalutamide is an AR inhibitor that acts on different steps in the AR signaling pathway. Enzalutamide has been shown to competitively inhibit androgen binding to ARs and inhibit AR nuclear translocation and interaction with DNA. The United States (US) Food and Drug Administration (FDA) approved Xtandi (enzalutamide) capsules in August 2012 based on a benefit in overall survival for men with metastatic castration-resistant prostate cancer (CRPC) who previously received docetaxel therapy (14). In men with prostate cancer, the maximum tolerated dose (MTD) was determined to be 240 mg daily, but after review of the safety and efficacy data available, the optimal dose of enzalutamide was determined to be 160 mg/day. More than 4200 subjects and patients have been enrolled worldwide in complete and ongoing clinical trials evaluating enzalutamide as of October 2012.

The most common adverse reactions (drug-related) ($\geq 5\%$) in patients treated with enzalutamide (N = 800) in the phase 3 study CRPC2 (AFFIRM) (N = 1199) were asthenia/fatigue, back pain, diarrhea, arthralgia, hot flush, peripheral edema, musculoskeletal pain, headache, upper respiratory infection, muscular weakness, dizziness, insomnia, lower respiratory infection, spinal cord compression and cauda equina syndrome, hematuria, paresthesia, anxiety, and hypertension (12). Discontinuations due to adverse events were reported for 16% of enzalutamide-treated patients and 18% of placebo-treated patients. The most common adverse event leading to treatment discontinuation was fatigue (0.6% of enzalutamide-treated patients and 0.3% of placebo-treated patients). The most common adverse reaction leading to treatment discontinuation was seizure, an identified risk and a targeted medical event, which occurred in 0.9% of the enzalutamide-treated patients and none (0%) of the placebo-treated patients.

The use of enzalutamide in women with breast cancer is under investigation. In the completed study, MDV3100-08 (NCT01597193), 160 mg/day was determined to be the recommended phase 2 dose. The PK and safety profile in women was found to be similar to that observed in men. The dose-expansion portion of this study found evidence of some activity of enzalutamide at 160 mg/day as a single agent in women with AR+ breast cancer in all subtypes of breast cancer. Enzalutamide was combined with either anastrozole or exemestane in women with ER+ breast cancer. Enzalutamide was found to be a potent CYP3A4 inducer and reduced the AUC of anastrozole by 80% and reduced the AUC of exemestane by about 50%. Double dose exemestane, ie, 50 mg/day was found to be safe in combination and provided full suppression of estradiol. This dosing was then used for a randomized double blind phase II trial of exemestane +/- enzalutamide in ER+ AR+ MBC Protocol MDV3100-12. This trial has been completed, but is currently maturing for efficacy endpoints. No new safety signals were found. In addition, the use of enzalutamide in women with advanced AR+ TNBC was evaluated in a single-arm, open-label phase 2 study, MDV3100-11 (NCT01889238).

Results have been reported at ASCO 2015. A trial of enzalutamide plus trastuzumab is ongoing in Her2+ AR+ BC. (See preliminary studies).

As part of the phase I study, enzalutamide was combined with full dose fulvestrant. Preclinical modeling showed synergistic inhibitory effects on tumor cell growth (13). Fulvestrant is not significantly metabolized by CYP3A4. In this substudy, 11 patients with ER+ AR+ BC had loading doses of fulvestrant 500 mg every 2 weeks for one month, then were treated with fulvestrant 500 mg IM every 4 weeks as standard of care (13). Open label enzalutamide at 160 mg po daily was added. PK was done for both drugs to look for interactions. The median age of these patients was 59 (53-78) years, and 64% had prior treatment for metastatic disease. No significant PK interaction was found and no new safety signals were found. Several patients remained on this combination treatment for over a year.

1.3 Preliminary Studies/Progress Report

Drs. Richer and Elias received a Department of Defense Clinical Translation Research Award to study the role of AR signaling and the effect of inhibiting that signal in breast cancer in both the preclinical and clinical setting. Given a long-standing relationship with Medivation, both Medivation and Astellas agreed to collaborate in these efforts. The current LOI addresses the need to obtain serial biopsies from patients receiving enzalutamide for breast cancer. We plan to do these clinical trials at multiple selected sites.

Preclinical modeling demonstrated that enzalutamide blocked the ability of AR to translocate to the nucleus with or without ligand (4-OH testosterone) and that this caused significant cytotoxicity both in vitro and in vivo in a variety of breast cancer cell lines and patient-derived xenografts (7). Despite the fact that enzalutamide does not bind ER, it blocked ER signaling and estradiol growth stimulation in ER+BC cells. Further laboratory investigation has now shown that AR binds directly with ER and regulates the DNA binding sites for ER (8).

Thus far, trials sponsored by Medivation and/or Astellas have established:

- Phase I evaluation of single agent enzalutamide (9, 10): PK of enzalutamide not different in women; RP2D 160 mg po daily. Toxicities similar to that of men – largely G1/2 fatigue, nausea, hot flashes, and occasional mild transaminitis. Because of concern over possible seizure risk (men with prostate cancer ~6/3000 had seizures, but most of these had had brain metastases or CNS surgery), women with brain metastases have been excluded from all enzalutamide trials.
- Phase I/PK studies (11-13): enzalutamide with anastrozole, single dose exemestane, double dose exemestane, and fulvestrant – AUC of anastrozole reduced by ~80%; AUC of exemestane reduced by ~50%; AUC of double dose exemestane with concurrent enzalutamide was equivalent to single dose exemestane when given alone; and the AUC of fulvestrant was not obviously affected by enzalutamide. Anti-tumor efficacy is documented in each of these trials.
- Phase II enzalutamide in AR+ TNBC (12): 29% clinical benefit rate at 24 weeks.

- Phase II randomized trial of double dose exemestane +/- enzalutamide in AR+ ER+ BC: results maturing.
- The phase I of fulvestrant/enzalutamide combination established safety and lack of PK interaction, but only included 11 patients (13). Preclinical modeling of this combination showed synergy (13).

1.3.1 Preliminary Data Points

Our preliminary data points to AR expression as being a prerequisite for targeting AR. For our studies, we have set a low bar for AR expression: positive is defined as 1% of cells staining with strong intensity (analogous to the Allred score for ER and PR). Clearly other cutoffs may be worth exploring. We define our outcome, lack of response, as no response or PFS<12 weeks (NR group). The following molecular characteristics at baseline will be explored among others: 1) nuclear to cytoplasmic localization of AR staining. 2) strength of AR signaling as measured by the expression of downstream AR-regulated genes, which should reflect dependence of the tumor cell on AR function. 3) AR:ER ratio with resistance to anti-estrogen therapy. The strength of other pathways signals that bypass the need for AR or ER signaling may be a harbinger for resistance to enzalutamide, hence our interest in examining the PI3K/mTOR pathway as that pathway integrates many of the signals emanating from other growth factor receptor pathway activations. These are expected findings, but we will also conduct unsupervised analyses to discover other potential associations. Because we will not reliably have normal breast tissue from the patients, we will not be able to compare gene expression and functional protein pathway activation mapping in the tumor vs. normal at baseline; hence, the analysis will be restricted to relative gene expression or protein expression or the presence of mutations in the tumor vs. the PFS. Adding strength to our analysis will be our ability to parallel the clinical tissue with preclinical tissue, and evaluate the most promising of the potential predictive biomarkers in both systems. Consistent and biologically plausible putative biomarkers would then require confirmation using more quantitative and specific assays.

1.4 Hypothesis

Enzalutamide with fulvestrant, by blocking both AR and ER signaling, will synergize in patients with ER+ AR+ metastatic breast cancer. It may prevent AR from taking over from ER when ER signaling is blocked. The extent of AR expression that correlates with enzalutamide efficacy is unknown and is an objective of this study; therefore, all patients, including those with “AR-negative” breast cancer (although this is expected to be fewer than 10%), will be enrolled to receive standard of care fulvestrant with enzalutamide. We hypothesize that 1) AR localization (a shift from nuclear to cytoplasmic) when comparing the pretreatment to the ~4 week tissues will represent a pharmacodynamics (PD) marker of enzalutamide activity. In tumors that respond to treatment, we would not only expect such a shift in AR localization, but also 2) a reduced expression in AR-regulated genes as well as subsequent changes in downstream processes. Thus, comparison of pre- vs during treatment tissues should allow us to identify PD markers as well as predictive biomarkers that indicate no response to enzalutamide (such as no

change in Ki67 or TUNEL). We will be able to measure Ki67 using standard IHC assays as well as a quantitative measurement by Reverse Phase Protein Microarray (RPMA). Multivariate analysis will be required to attempt to classify protein, gene, or TUNEL markers associated with PFS as predictive (of therapeutic impact), prognostic (of intrinsic tumor biology), or mixed as discussed above. A similar plan to compare the preclinical with the clinical analyses should facilitate the discovery and validation of putative biomarkers. The evaluation of Ki67 and TUNEL will provide the preliminary data necessary to mount our preoperative trial proposed in Years 3-5. Sample size and power consideration: 24 baseline samples will provide 80% power to detect a mean difference in change (baseline-during treatment) of 1 (or 1.3) standard deviation between the two groups (NR vs R) using one-sided 2-group t-test with a significance level of 0.05, assuming the response is 50% (or 20%). Statistical analysis plan: the same analysis approaches described in aim 1 will be used here, with the changes (baseline –during treatment) instead of the baseline values. Coefficient variation (CV) will be calculated for each potential biomarkers and biomarkers with smaller CV will be particularly interesting.

2. OBJECTIVES AND OUTCOMES:

2.1 Hypotheses and Specific Aims

This phase II study is designed to evaluate the tolerability and clinical activity of adding enzalutamide to fulvestrant treatment in women with advanced breast cancer that is ER positive and Her2 normal.

2.2 Primary Objective

To determine the clinical benefit rate (complete and partial response plus stable disease for 24 weeks) of the combination of enzalutamide/fulvestrant.

2.3 Secondary Objectives

1. To confirm the safety profile of the combination.
2. Response rate (complete and partial response) at 24 weeks, Progression-free survival (PFS) and % of patients remaining free from progression at 12 and 24 weeks.
3. To obtain serial biopsies of breast cancer pretreatment, during treatment and at time of tumor progression in order to determine the extent of AR expression and signaling in breast tissue, to evaluate the effect of enzalutamide on the tumor, and to evaluate the relationship of these effects on clinical outcomes.

2.4 Laboratory Hypotheses (From Serial Tissue Biopsies)

1. In the luminal (ER+/AR+) tumors, decrease in Ki67 after ~4 weeks of treatment to below 10% is associated with response to therapy and will correlate with progression-free survival (PFS).
2. Pretreatment molecular characteristics (such as AR:ER ratio in ER+ tumors, Her2 status, PI3K pathway mutations, or others based on further profiling of sensitive versus resistant cell line and patient samples) will be associated with lack of response and/or prolonged PFS.

3. Tissue at time of disease progression will be enriched for genes/proteins representing resistance mechanisms. Timing of such progression (early vs. late) may yield different hypotheses for resistance, and resistance mechanisms will likely vary with subtype, since we already have found that the mode of action by which enzalutamide inhibits tumor growth differs in ER+/AR+ as compared to ER-/AR+ tumors.

3. STUDY DESIGN AND RESEARCH METHODS:

3.1 Study Design

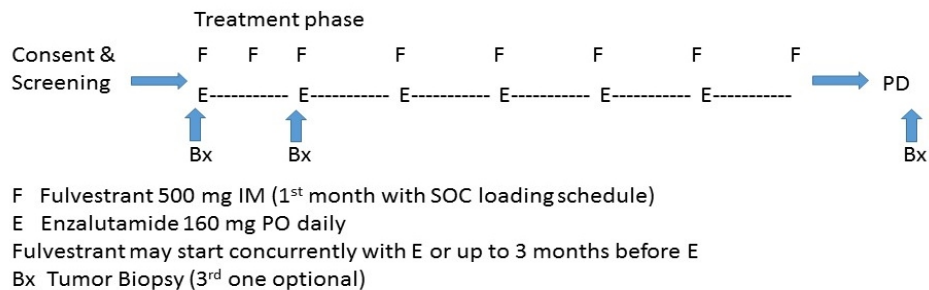
Single arm, non-randomized, open-label treatment consisting of:

- Fulvestrant 500 mg IM given days 1, 15, 28, then every 4 weeks as per standard of care (SOC). Fulvestrant may have already been started.
- Enzalutamide 160 mg daily.

If pre- or perimenopausal, patients will also receive goserelin 3.6 mg sq every 4 weeks (or equivalent) as per standard of care (SOC).

3.2 Study Schema

Phase II trial of fulvestrant plus enzalutamide in ER+/Her2- advanced breast cancer



3.3 Study Periods

The study periods will be as follows for all patients:

- Screening: Up to 28 days before treatment;
- Treatment: Day 1 of treatment through discontinuation of enzalutamide treatment;
- Safety follow-up: Approximately 30 days after the last dose of enzalutamide or before initiation of a new antitumor treatment, whichever occurs first.

3.4 Sample Size

Twenty-four (24) evaluable patients will be recruited for this study. Up to 45 patients may be consented to allow for screen failures. The 24 evaluable patients will receive serial

tumor biopsies – pre-enzalutamide treatment and at ~4 weeks on therapy. Patients will be asked to allow an optional biopsy upon progression of disease.

4. STUDY ENROLLMENT AND WITHDRAWAL:

4.1 Inclusion Criteria

1. ER+ Her2- breast cancer
2. Metastatic
3. Female, at least 18 years of age
4. Candidate for fulvestrant therapy – patients who have started fulvestrant may enter this trial if within 3 months of starting fulvestrant
5. Measurable or evaluable by RECIST 1.1
6. ECOG PS 0-2
7. Able to swallow study drug and comply with study requirements
8. Tumor available for fresh biopsy (two biopsies – pretreatment as regards enzalutamide, and during treatment at 4 weeks). The patient will be also be asked if they would be willing to provide a third biopsy at time of progression.
9. If patient is pre- or peri- menopausal, then will need to have concurrent ovarian suppression. Patients may have already gotten the loading dose of ovarian suppression. Pre- or peri- menopausal subjects must have a negative urine pregnancy test confirmed at screening.
10. ANC >1000/uL and platelets >75,000/uL at screening visit
11. Total bilirubin < 1.5 times upper limit of normal (ULN) at the screening visit unless an alternate nonmalignant etiology exists (eg, Gilbert's disease)
12. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) < 3 times ULN or < 5 times ULN if patient has documented liver metastases
13. Creatinine < 1.5 times ULN
14. INR < 1.5 times ULN, or if on warfarin, can safely transition off for biopsy
15. Willing to donate blood for research at 4 time points
16. Written informed consent obtained prior to biopsies and blood samples
17. Agreement to exercise appropriate use of contraception. Subjects should use 2 acceptable methods of birth control (one of which must include a condom as a barrier method of contraception) starting at the time of screening for an enzalutamide study and continuing throughout the course of treatment and for at least three months after enzalutamide is discontinued.

4.2 Exclusion Criteria

1. Current or previously treated brain or leptomeningeal metastases
2. History of seizures

3. Prior treatment with an anti-androgen (abiraterone, ARN-509, bicalutamide, enzalutamide, ODM-201, TAK-448, TAK-683, TAK-700, VT-464)
4. Systemic estrogens or androgens within 14 days before initiating therapy. Vaginal estrogens are allowed if necessary for patient comfort.

4.3 Withdrawal

The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

An investigator may also terminate a participant's enrollment in the study if any clinical adverse event, laboratory abnormality, non-compliance, lost to follow-up, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.

5. STUDY AGENT:

5.1 Enzalutamide

5.1.1 General Information

The study drug, enzalutamide, is approved in the US to treat men with metastatic CRPC who previously received docetaxel.

5.1.2 Enzalutamide Product Characteristics

Enzalutamide, also known as MDV3100, has the chemical name 4-{3-[4-cyano-3-(trifluoromethyl)phenyl]-5,5-dimethyl-4-oxo-2-sulfanylideneimidazolidin-1-yl}-2-fluoro-*N*-methylbenzamide. The drug substance is formulated in the surfactant caprylocaproyl polyoxylglycerides, also known as Labrasol. The product will be supplied as white to off-white soft gelatin capsules containing 40 mg of enzalutamide.

5.1.3 Enzalutamide Packaging

Enzalutamide is packaged in bottles with induction-sealed child-resistant caps labeled with the study protocol number, contents, directions for use, storage directions, clinical trial statement, and sponsor name. Each bottle contains 120 capsules (30-day supply).

5.1.4 Enzalutamide Storage

Enzalutamide study drug is to be handled and stored safely and properly in accordance with the study drug label.

5.1.5 Directions for Administration of Enzalutamide

The daily dose of enzalutamide is 160 mg/day in 4 capsules (40 mg each) given by mouth. Patients should self-administer enzalutamide study drug by mouth once daily, with or without food, starting on day 1. The capsules should be swallowed whole without chewing, dissolving, or opening them. Patients will self-

administer the study drug at home. Patients should not make up missed or vomited doses; dosing should resume on the next calendar day unless otherwise instructed.

5.1.6 Directions for Dose Modification of Enzalutamide

Patients who experience a grade 3 or higher toxicity that is attributed to enzalutamide study drug and cannot be ameliorated by the use of adequate medical intervention may interrupt treatment with enzalutamide. Subsequently, the enzalutamide dosing may be restarted at the original dose (160 mg/day, 4 capsules) or a reduced dose (80 mg/day, 2 capsules). Treatment interruption and re-initiation should be discussed with the principal investigator.

5.1.7 Treatment Compliance

Study drug accountability will be performed to document compliance with the dosing regimen. Patients will be asked to return all bottles of study drug at study visits. Patients who forget to return bottles will be asked to return them at the next study visit.

5.1.8 Drug Inventory and Accountability

Drug inventory and accountability records for the study drugs will be kept by the investigator/pharmacist. Used or unused study drug may be destroyed at the study site according to standard institutional procedures if the sponsor agrees with the procedure, and after drug accountability has been conducted by the sponsor or representative, unless otherwise approved.

5.1.9 Effects of Enzalutamide on Exposure to Other Drugs

Clinical data indicate that enzalutamide is a strong inducer of CYP3A4 and a moderate inducer of CYP2C9 and CYP2C19. Concomitant use of enzalutamide with drugs with a narrow therapeutic index that are metabolized by CYP3A4 (eg, alfentanil, cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus, tacrolimus), CYP2C9 (eg, phenytoin, warfarin), and CYP2C19 (eg, S-mephenytoin) should be avoided if possible as enzalutamide may decrease their exposure. If coadministration with warfarin cannot be avoided, additional INR monitoring should be conducted.

5.1.10 Drugs That May Affect Exposure to Enzalutamide

5.1.10.1 Drugs That Inhibit or Induce CYP2C8

Coadministration of a strong CYP2C8 inhibitor (eg, gemfibrozil) increased the composite AUC_{0-∞} of enzalutamide plus its active metabolite in healthy volunteers by 2.2-fold; therefore, coadministration of enzalutamide with strong CYP2C8 inhibitors should be avoided if possible. If coadministration of enzalutamide with strong CYP2C8 inhibitors cannot be avoided, the enzalutamide dose should be reduced to 80 mg once daily. If coadministration of the strong inhibitor is discontinued, the enzalutamide dose should be returned to the dose used prior to initiation of the strong CYP2C8 inhibitor.

The effects of CYP2C8 inducers on the PK of enzalutamide have not been evaluated in vivo. Coadministration of enzalutamide with strong or moderate CYP2C8 inducers (eg, rifampin) may alter the plasma exposure of enzalutamide and should be avoided if possible. Selection of a concomitant medication with no or minimal CYP2C8 induction potential is recommended.

5.1.10.2 Drugs That Induce CYP3A4

The effects of CYP3A4 inducers on the PK of enzalutamide have not been evaluated in vivo. Coadministration of enzalutamide with strong CYP3A4 inducers (eg, carbamazepine, phenobarbital, phenytoin, rifabutin, rifampin, rifapentine) may decrease the plasma exposure of enzalutamide and should be avoided if possible. Selection of a concomitant medication with no or minimal CYP3A4 induction potential is recommended. Moderate CYP3A4 inducers (eg, bosentan, efavirenz, etravirine, modafinil, nafcillin) and St. John's Wort may also reduce the plasma exposure of enzalutamide and should be avoided if possible.

5.1.10.3 Precautions Regarding Concomitant Medications

Refer to the following websites for updated lists of CYP inhibitors, inducers, and substrates.

- <http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#potency>
- <http://medicine.iupui.edu/clinpharm/ddis/table.aspx>

5.2 Fulvestrant

Fulvestrant is an ER antagonist indicated for the treatment of postmenopausal women with hormone receptor-positive advanced breast cancer. Unlike the selective ER modulator tamoxifen, which displays mixed antagonist/agonist properties, fulvestrant has no agonist effects. Fulvestrant binds, blocks, and increases degradation of the ER, leading to an inhibition of estrogen signaling. This distinctive mode of action may overcome tumor resistance to previous endocrine agents. The increased degradation of the ER by fulvestrant may also limit cross-talk between growth factor receptor and ER-mediated signaling pathways and thus increase the duration of response observed with fulvestrant treatment.

Per the USPI8, the recommended dose of fulvestrant is 500 mg administered by IM injection on days 1, 15, and 29 and once monthly thereafter. The most common clinically significant adverse reactions occurring in $\geq 5\%$ of patients receiving fulvestrant 500 mg are injection site pain, nausea, bone pain, arthralgia, headache, back pain, fatigue, pain in extremity, hot flush, vomiting, anorexia, asthenia, musculoskeletal pain, cough, dyspnea, and constipation. In addition, \geq grade 1 elevations in AST, ALT or alkaline phosphatase were observed in $> 15\%$ in pooled analysis of fulvestrant trials, with grade 3 to 4 increases observed in 1% to 2% of patients. Clinical data show that fulvestrant does not affect the pharmacokinetics of midazolam (a sensitive CYP3A4 substrate) and rifampin and

enzalutamide (a potent CYP3A4 inducer) had no effect on the pharmacokinetics of fulvestrant.

5.3 Investigational Product Accountability

The Investigator must maintain accurate records (including dates and lot numbers) of all study drug supplies received. All study drug supplies issued to, used by, and returned by each patient must be recorded on a Drug Accountability Log completed by the Investigator or designee. All remaining study supplies, opened or unopened, must be returned to the Sponsor (or designee) at the end of the study or destroyed on site according to the site's standard operating procedures only after study drug accountability has been completed and with approval of the Study Monitor. All records must be made available to the Sponsor (or designee) and appropriate regulatory agencies upon request.

6. STUDY PROCEDURES AND SCHEDULE:

6.1 Study Procedures

Study Phase	Screening	Treatment				Progression	Follow-up
		1	5	9	13 [1]		
Study Week	-4 to -1	1	5	9	13 [1]	varies	varies [10]
Window (Days)	28	n/a	+/- 7	+/- 7	+/- 7	n/a	+/- 7 days
Informed Consent	x						
Eligibility	x						
Medical History	x						
Archived Tissue Collection [2]	x						
Tumor Imaging [3]	x			x			
Physical Exam	x	x	x	x	x	x	
Adverse Events	x	x	x	x	x	x	x
Concomitant Medications	x	x	x	x	x	x	
Study Drug Dispensing [4]		x		x			
Fulvestrant [9]		x	x	x	x		
Tumor Biopsy		x [5]	x			optional	
Hematology, Serum Chemistry [6]	x	x [8]	x	x	x	x	
PT/INR, PTT [11]	x		x			x	
Urine Pregnancy Test [12]	x						
Circulating Tumor Biomarkers	x	x [8]	x	x	x	x	
CTCs [7]	x	x [8]	x	x		x	
Survival & Recurrence Follow-up							x

[1] Continue every 4 weeks until progression.

[2] From primary lesion and/or metastatic sites previously biopsied. If tissue is available, archival blocks from both sites (primary and metastatic) should be collected, however, the top priority is the primary tissue.

[3] Within approximately 28 days before study entry and includes CT with contrast (unless medically contraindicated) of chest, abdomen, and pelvis. Bone assessments as clinically indicated. Thereafter, perform assessments every 8 weeks up to week 49 and then every 12 weeks thereafter until progression is documented per RECIST 1.1 or permanent treatment discontinuation. MRI is acceptable if CT not possible. Use the same testing across time.

[4] Refill every 8 weeks as long as patient remains on active treatment.

[5] Before treatment, but after eligibility determined.

[6] Hematology: hematocrit, hemoglobin, platelet count, red blood cell count, total neutrophils (absolute), white blood cell count with differential. Chemistry: albumin, alkaline phosphatase, ALT (alanine aminotransferase), AST (aspartate transaminase), blood urea nitrogen and creatinine; Ca⁺⁺, Cl⁻, K⁺, Na⁺; glucose (non-fasting), total bilirubin, total CO₂ (bicarbonate), total protein.

[7] CTCs will be collected at all participating sites, coded to remove patient identifiers, and shipped to UCCC and USC for processing as outlined in the lab manual.

[8] Only one sample is needed pretreatment (either during screening or prior to treatment on C1D1).

[9] Given IM on days 1, 15, and 28 (C2D1), and then every 4 weeks as per standard of care (SOC).

[10] 30 days after the last dose of enzalutamide or before initiation of a new antitumor treatment, whichever occurs first. May be done by telephone.

[11] Collected at screening, tumor biopsy time points if clinically required for biopsy, and as required per institutional standard.

[12] If woman of child bearing potential only.

Bold represents non-SOC events.

6.2 Drug Dispensing

An 8 week supply of enzalutamide will be provided to each patient at a time (provided by Astellas/Medivation) and dispensed by the research pharmacy. Additional bottles may be dispensed to accommodate necessary windows for subsequent visits or for accidental wastage.

6.3 Laboratory Procedures/Evaluations

6.3.1 Blood

Routine blood tests (chemistries, hematology) will be obtained every month as clinically indicated. Tumor markers to be followed every 1-2 months as clinically indicated.

Blood for potential pharmacogenomics testing will be obtained at baseline. The white cell pellet required for this sample will be obtained from the baseline plasma vacutainer.

Plasma for tumor related exosomes and other circulating tumor products will be obtained each cycle on day 1 (10 ml).

Blood for circulating tumor cells will be obtained day 1 of cycles 1, 2, and 3 (up to 30ml each time).

Baseline:

- 1 plasma vacutainer (green top, lithium heparin, 10ml)
- 1 serum vacutainer (red top, 10ml)
- 2-3 tubes for CTC sample collection (up to 30ml)

~Week 5:

- 1 plasma vacutainer (green top, lithium heparin, 10ml)
- 2-3 tubes for CTC sample collection (up to 30ml)

~Week 9:

- 1 plasma vacutainer (green top, lithium heparin, 10ml)
- 2-3 tubes for CTC sample collection (up to 30ml)

~Week 13:

- 1 plasma vacutainer (green top, lithium heparin, 10ml)

End of study:

- 1 plasma vacutainer (green top, lithium heparin, 10ml)
- 2-3 tubes for CTC sample collection (up to 30ml)

6.3.2 Serial Biopsies

At each time point described above, subject will have a biopsy obtained (up to 3 cores), divided into a formalin fixed, paraffin embedded specimen and a fresh frozen specimen to be analyzed. Biopsy can be either needle biopsy or excisional biopsy, depending on the site of the tumor, according to physician's

preference. Tissue will be used for future studies, if subjects consent to tissues to be banked. See study procedure manual for shipping and other instructions. The purpose of the baseline pre-enzalutamide tumor tissue is to gather an understanding of ER+ breast cancers that may or may not benefit from AR signaling inhibition. The biopsy 4 weeks into treatment is taken at the time that both drugs are at their steady state concentrations (eg, following the loading doses of fulvestrant, and at least 4 half-lives of exposure to enzalutamide (T1/2 = 5.7 days). This will examine the subacute effects of this treatment on the tumor tissue, on AR and ER signaling. The purpose of the biopsy at time of progression is to obtain insights as to the mechanism of resistance to this treatment. We expect about half the patients to allow us to do this biopsy (based on previous experience).

7. RISKS AND BENEFITS:

7.1 Risks of This Protocol

- Clinical toxicities of fulvestrant (standard agent) plus enzalutamide as outlined in package insert and investigator brochure.
- Biopsies (each one is requested of the patient).
- Misuse or inadvertent release of information.
- Identification of the patient by release of comprehensive genomic information.
 - Pharmacogenomic analysis typically does not identify a genetic predisposition to a disease since its analysis typically is restricted to enzymes that metabolize the therapeutically administered agents.
- Low white blood cell count, which may increase risk of infection.
- Blood clot in the lungs (also known as pulmonary embolism).

7.2 Knowledge to Be Gained

- The relationship between the clinical efficacy measures (response, clinical benefit rate, PFS) and AR signaling will be examined.
- Characteristics of breast cancers that are resistant to enzalutamide with or without other targeted agents. A set of putative biomarkers will be identified.
- Mechanisms of resistance to enzalutamide-based treatment that arise in the clinic.

7.3 Justification of Risks

The clinical impact of enzalutamide in breast cancer is not yet determined. If the tumor appears to progress, the patient can be taken off study and treated accordingly as per the treating physician.

The extent of AR expression that correlates with enzalutamide efficacy is unknown and is an objective of this study; therefore, all patients, including those with “AR-negative” breast cancer, will be enrolled to receive standard of care fulvestrant with enzalutamide.

The choice of this combination was to take advantage of the lack of PK interaction between the two agents and conduct a phase II trial.

8. ASSESSMENT OF SAFETY:

8.1 Serious Adverse Events

A serious adverse event or reaction is any untoward medical occurrence that at any dose:

- Results in death (death solely as a result of disease progression is not a serious adverse event);
- Is life threatening (i.e., the patient was at immediate risk of death at the time of the event). "Life threatening" does not include an event that hypothetically might have caused death if it were more severe. For example, drug induced hepatitis that resolved without evidence of hepatic failure would not be considered life threatening even though drug induced hepatitis can be fatal;
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity (i.e., a substantial disruption of the patient's ability to carry out normal life functions);
- Is a congenital anomaly/birth defect;
- Is a medically important event, including seizure.

8.2 Serious Adverse Event Reporting

NCI CTCAE 4.03 will be used for adverse event reporting.

Within 24 hours of awareness of a serious adverse event, whether or not related to the study drug, the Investigator or delegated personnel will complete and submit a Medwatch 3500A Form to the sponsor investigator and Astellas, containing all required information (reference 21 CFR 312.32). The sponsor investigator will submit the SAE to any additional regulatory authorities as required. If submission of this SAE to the sponsor investigator or Astellas is not possible within 24 hours, the Investigator's local drug safety contact (IRB, etc.) should be informed by phone.

The PI will record all reportable events with start dates occurring any time after informed consent is obtained. Non-serious AEs will be recorded until 30 days after the last day of study treatment or until initiation of new therapy, whichever comes first. SAEs will be followed until resolution or stabilization.

Notification of the Principal investigator, Dr. Anthony Elias:

Anthony.elias@ucdenver.edu

Cell 303-638-2018

ACP 5310, MS 8117

1665 North Aurora Court
Aurora, CO 80045

The SAE documentation, including the Medwatch 3500A Form and available source records should be emailed or faxed to:

Astellas Pharma Global Development – United States
Email: Safety-us@us.astellas.com
Fax number: (847) 317-1241

The following minimum information is required:

- Study number/IIT regulatory identifier
- Subject number, sex and age
- The date of report
- A description of the SAE (event, seriousness of the event)
- Causal relationship to the study drug

Follow-up information for the event should be sent promptly (within 7 days) as necessary.

The Principal Investigator, Dr. Anthony Elias, will ensure prompt reporting of the SAE to all investigators and co-PIs of the study.

8.3 Adverse Events

- An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation patient administered a pharmaceutical product and which does not necessarily have to have a causal relationship with study drug.
- An adverse event observed after starting administration of the study drug or comparator drug is called a “treatment-emergent adverse event.” Treatment-emergent adverse events will be analyzed and discussed in the clinical study report for this study.

8.4 Procedure in Case of Pregnancy

- The effect of enzalutamide in pregnant and lactating women is not known, and the exposure of a fetus or nursing infant is considered a potential risk. Enzalutamide can cause fetal harm when administered to a pregnant woman based on its mechanism of action. Subjects receiving enzalutamide are advised to use 2 acceptable methods of birth control (one of which must include a condom as a barrier method of contraception) starting at the time of screening for an enzalutamide study and continuing throughout the course of treatment and for at least three months after enzalutamide is discontinued.
- The Investigator should report the outcome of the pregnancy (independent of outcome, eg. full term delivery, pre-term delivery, spontaneous abortion, induced abortion, stillbirth, death of newborn, congenital anomaly [including anomaly in a

miscarried fetus, etc.] in accordance with the same reporting procedure as for SAEs. The date of outcome of the pregnancy, gestational age, date of birth and neonatal data etc., should be included in this information.

8.5 In Case of Injury

In the event of a side effect or injury, appropriate medical care as determined by the Investigator or his/her designated alternate will be provided. No other compensation of any type will be provided by the study Sponsor.

8.6 Safety Monitoring and Reporting

A monthly teleconference will be held amongst the institutional investigators to review patients and adverse events.

9. STUDY OVERSIGHT:

9.1 Data Safety and Monitoring Oversight

The sponsor investigator will be responsible for monitoring the trial per the trial monitoring plan, in addition to overseeing the safety and efficacy of the trial including any specimens collected, executing the data and safety monitoring (DSM) plan, and complying with all reporting requirements to local and federal authorities. This oversight will be accomplished through additional oversight from the Data and Safety Monitoring Committee (DSMC) at the University of Colorado Cancer Center (CU Cancer Center). The DSMC is responsible for ensuring data quality and study participant safety for all clinical studies at the CU Cancer Center, which is the coordinating institution of this trial.

A summary of the DSMC's activities is as follows:

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

Per the CU Cancer Center Institutional DSM Plan, SAEs, UAPs and reportable AEs are reported to the DSMC, IRB and the sponsor investigator per protocol. All SAEs, UAPs and reportable AEs are to be reported to the DSMC within 5 business days of the sponsor investigator receiving notification of the occurrence.

Each subject's treatment outcomes will be discussed by the site PI and appropriate staff at regularly scheduled meetings. Data regarding number of subjects, significant toxicities, dose modifications, and treatment responses will be discussed and documented in the meeting's minutes.

The sponsor investigator is responsible for organizing and conducting regularly scheduled teleconferences with all participating sites. The sponsor investigator will also be responsible for including data from all of the participating sites to include the minutes from these regularly scheduled teleconferences between the sponsor investigator and the sites within the overall trial's six month DSM report.

The sponsor investigator will provide a DSM report to the CU Cancer Center DSMC on a six month basis. The DSM report will include a protocol summary; current enrollment numbers; summary of toxicity data to include specific SAEs, UAPs and AEs; any dose modifications; all protocol deviations; and protocol amendments. The DSM report submitted to the DSMC will also include, if applicable, the results of any efficacy data analysis conducted. Results and recommendations from the review of this six month report by the DSMC will then be provided to the sponsor investigator in a DSMC review letter. The sponsor investigator is then responsible for ensuring this letter is submitted to the site's IRB of record at the time of IRB continuing review.

9.2 Clinical Monitoring

Clinical site monitoring will be conducted to ensure that the rights and well-being of human participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/ amendment(s), with GCP, and with applicable regulatory requirement(s).

The Research Monitor, Elaine Lam, MD is responsible to oversee the safety of the research and report observations/findings to the IRB or a designated institutional official. The Research Monitor will review all unanticipated problems involving risks to subjects or others associated with the protocol and provide an independent report of the event to the IRB. The Research Monitor may discuss the research protocol with the investigators; shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report; and shall have the responsibility to promptly report their observations and findings to the IRB or other designated official and the HRPO. Dr. Lam is a member of the University of Colorado Cancer Center and is a medical oncologist specializing in prostate cancer. She is a member of the UCCC Data Monitoring and Safety Committee.

Monitoring for this study will be performed by CU Cancer Center Clinical Monitor in accordance with the clinical monitoring plan (CMP), incorporated herein by reference. The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of the monitoring reports. As necessary, requests for data clarification or correction will be sent to the appropriate site PI.

9.3 Auditing

Independent audits will be conducted by the CU Cancer Center DSMC. Independent auditors from the sponsor investigator's authorized representative will be allowed by the site's PI. In addition, audits may be conducted at any time by appropriate regulatory authorities and/or the IRB. During the course of the study and after it has been

completed it is likely that 1 or more study site visits will be undertaken by authorized representatives of the Sponsor.

The purpose of the audit is to ensure that the study is being, or has been, conducted and monitored in compliance with the protocol as well as recognized cGCP guidelines and regulations, to ensure monitoring practices are performed consistently across all participating sites and that monitors are following the CMP, as defined herein. These audits will also increase the likelihood that the study data and all other study documentation can withstand a subsequent regulatory authority inspection.

If such audits are to occur, they will be arranged for a reasonable and agreed time.

All reports and patient samples will be identified only by the patient's ID number and the patient's initials in order to maintain patient confidentiality. Additional patient confidentiality issues are covered in the Clinical Trial Agreement and in the informed consent form signed by the patient.

10. STATISTICAL CONSIDERATIONS:

10.1 Clinical Data

Age, ethnicity, race, gender, breast cancer histology (ductal, lobular, other), breast cancer subtype (ER, PR, Her2, Ki67, AR), detailed prior treatment history, selected comorbidities (especially insulin, metformin use), clinical response, CBR, PFS at 12 weeks, TTP. Details of treatment, including AE, SAE, and compliance.

10.2 Sample Size

10.2.1 Determination of Sample Size

The primary outcome is the clinical benefit rate. Assuming the undesirable rate of 10% and desirable rate of 30%, a sample size of 24 provides 89% power to detect this 25% rate difference using an exact binomial test with a one-sided alpha of 0.085. If 5 or more patients show clinical benefit the drug combination warrants further evaluation. During treatment period, patients might withdraw due to toxicity or loss to follow-up before on treatment for 24 weeks. Table below summarize the statistical power and the decision rule for sample size from 24 to 20, a 20% loss in the total sample size, which is the upper bound for loss seen in practice.

Statistical Power	n	Actual Alpha	Reject H0 If R ≥
0.89	24	0.085	5
0.86	23	0.073	5
0.84	22	0.062	5
0.80	21	0.052	5
0.76	20	0.043	5

R is the response as the clinical benefit

10.2.2 Power Consideration

For the outcome lack of response, 24 samples gathered during years 1-2 provide 80% power to detect a minimum mean difference at baseline of 1.2 (or 1.5) standard deviation between the NR group vs all others (Response group, R), using a two-sided t-test with a significance level 0.5, assuming 50% (or 20%) response rate. Due to the exploratory nature of this aim, the type I error rate will not be adjusted for exploring multiple biomarkers.

10.3 Research Laboratory Data

AR IHC quantitation, AR pathway activation, AR localization (nuclear/cytoplasmic), Ki67 and response, cleaved caspase 3, AR mRNA, AR mutational status, AR splice variants, ER IHC quantitation, ER mRNA, RNAseq, whole exome sequencing, proteomic analysis.

10.4 Research (Statistical) Methods

Data collected in this study will be presented using summary tables, patient data listings, and figures. Disease evaluations will be performed by investigators per the RECIST ___ criteria. Each enrolled patients will provide tumor tissues at baseline (0 week) and during treatment (4 weeks) and at disease progression. 24 pre- and treatment- biopsy pairs are planned for this study. Patients lacking pre- or treatment- biopsies will be replaced.

10.4.1 Analysis Populations

Patients who received at least 4 weeks of enzalutamide treatment will be included in the efficacy analyses. Patients who received at least one dose of enzalutamide will be included for the safety evaluation.

10.4.2 Analysis Methods

10.4.2.1 Summary Statistics

Summary statistics will be used for the primary outcome clinical benefit rate as well as the response rate. Clinical benefit rate is defined as the proportion of subjects with complete response or partial response or stable disease. Response rate is defined as the proportion of subjects with complete response or partial response. 95% exact confidence intervals will be provided. Kaplan-Meier product limit survival plots will be generated and median survival time and associated 95% CI will be estimated, the PFS percentage at 12 and 24 weeks will be calculated. Descriptive statistics will be used to summarize the safety data such as the DLT for both hematologic and non-hematologic toxicity per type and grade, as well as all collected AE data, which will be listed per patient, and cycle. For the biopsy samples, exploratory analyses such as multivariable logistic regression, linear regressions and survival analyses will be required to attempt to classify protein, gene, or TUNEL markers associated with clinical outcomes such as response, clinical benefit and PFS as predictive (of therapeutic impact) or prognostic (of intrinsic tumor

biology). Bio-informatics tools such as unsupervised pathway analysis will also be explored. The following molecular characteristics at baseline will be explored among others: 1) Nuclear to cytoplasmic localization of AR staining. 2) Strength of AR signaling as measured by the expression of downstream AR-regulated genes, which should reflect dependence of the tumor cell on AR function. 3) AR:ER ratio with resistance to anti-estrogen therapy.

The same summary statistics will be used for the response rate and clinical benefit rate. Response rate is defined as the proportion of subjects with complete response or partial response. Clinical benefit rate is defined as the proportion of subjects with complete response or partial response or stable disease. Kaplan-Meier product limit survival plots will be generated and median survival time and associated 95% CI will be estimated for each arm. Combined and separate data for patients with AR+ and AR- BC and those with one prior endocrine therapy and those with more than one prior endocrine therapy for metastatic breast cancer.

10.4.2.2 Descriptive Statistics

Descriptive statistics will be used to summarize the DLT for both hematologic and non-hematologic toxicity per type and grade. The same summary statistics will be used for the response rate and clinical benefit rate. Response rate is defined as the proportion of subjects with complete response or partial response. Clinical benefit rate is defined as the proportion of subjects with complete response or partial response or stable disease. Kaplan-Meier product limit survival plots will be generated and median survival time and associated 95% CI will be estimated for each arm.

Descriptive statistics will be used to summarize the AEs and SAEs for both hematologic and non-hematologic toxicity per type and grade. Safety follow-up: Approximately 30 days after the last dose of both study drugs or before initiation of a new antitumor treatment, whichever occurs first.

10.5 Data Analysis Plan

10.5.1 Toxicity

The study populations will include the following:

- The Safety Population, which will include all patients who received at least 1 dose of enzalutamide;
- The Response Population, which will include all patients who received enzalutamide.

10.5.2 Response & Progression Free Survival (PFS)

10.5.2.1 Tumor Response

Tumor response will be assessed using RECIST 1.1.

10.5.2.2 Overall Response, Target Lesion Response, Non-Target Lesion Response:

Overall Response: If measurable disease, then confirmed by repeat assessments 4 or more weeks after initial documentation of the response.

If evaluable but not measurable, response will be assessed either quantitatively, in the case of patients with elevated tumor markers, or descriptively (e.g., resolution of bone scan findings, decrease in tumor related pain symptoms).

10.5.2.3 Progression Free Survival

For all patients enrolled, PFS will be defined as the time from the first day of enzalutamide treatment (Study Day 1) until documented disease progression or death on study, whichever occurs first. For patients who do not die or experience disease progression on study, PFS will be right censored at the day of the last information available for progression assessment. Kaplan-Meier methods will be used to describe PFS by dose level and overall.

10.5.3 Receptor Expression

The degree of ER, PR, and AR expression in the nucleus will be reported by IHC strength of staining (0-3+) and % nuclei stained. The relationship between tumor response and degree of AR expression, and/or AR signaling, will be examined. Additional assays for AR signaling, (e.g., gene expression array analyses, mutation analyses, or other immunohistochemistry evaluations) may be performed on remaining tissue to evaluate for molecular phenotypes and patterns associated with activated AR signaling.

11. SOURCE DOCUMENTATION AND RETENTION OF RECORDS:

11.1 Data and CRFs

The clinical data will be collected on eCRFs and stored in a secure, encrypted RedCap file.

11.2 Data Accessibility and Record Keeping

The Investigator must make study data accessible to the Study Monitor or other authorized representatives of the Sponsor (or designee) and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each patient must be maintained that includes the signed informed consent form and copies of all source documentation related to that patient. The Investigator must ensure the reliability and availability of source documents from which the information on the case report form was derived.

Investigators must maintain all study documentation for a period of 2 years following the approval date of the drug in breast cancer, or until 2 years after the investigational drug program is discontinued. Study documentation includes the Investigator's Brochure, signed protocol and amendments; signed informed consents; notifications of serious

adverse events and related reports; any dispensing and accountability logs; shipping records of investigational product and trial related materials; documentation of the financial aspects of the trial, insurance statement, and signed agreement between involved parties; dated and documented IRB approval, and approval of regulatory authority(ies); normal laboratory values; decoding procedures for blinded trials; initiation visit report; curricula vitae; and all correspondence pertaining to the conduct of the study. The Sponsor will notify the Study Investigator when any records may be discarded.

12. POTENTIAL SCIENTIFIC PROBLEMS:

12.1 Enzalutamide may not have sufficient anti-breast cancer activity in the clinic

Subsequent studies would then focus on defining the tumor characteristics that most likely would be sensitive to this or other AR signaling inhibitors and identifying rational combinations with enzalutamide to overcome this intrinsic resistance.

12.2 Inadequate collection of tissues and inadequate serial samples

We anticipate inadequate specimens in up to 10% of individuals. Each investigator is determined to collect these samples and is experienced in obtaining such specimens. Handling of these tissues is routine in all institutions. All participating sites are experienced NCI designated cancer centers, and have well developed phase I and breast cancer multidisciplinary programs. All sites see a large number of new breast cancer patients per year, and have high clinical trial accruals. All of the sites lead investigators are accomplished medical oncologists with experience with phase I/II and III trials. Dr. Elias in collaboration with Dr. Richer, conducted the preoperative hormone trial that established the potential importance of AR and enzalutamide in ER+ breast cancer. University of Colorado has well-established core facilities to perform Nextgen sequencing, tissue banking and processing, and CLIA certified IHC.

12.3 Inadequate tissue biopsy samples in more than 10% of individuals

We would then consider increasing the patient enrollment to obtain 20 matched serial samples (pre-enzalutamide and after 4 weeks).

13. ETHICS:

The PI will ensure that this study is conducted in full conformity with regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56. ICH E6 may also be followed to the extent it has been adopted by and is in accordance with FDA regulations.

14. CONFLICT OF INTEREST:

Independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Any actual conflict of interest of persons who have a role in

the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed by the University of Colorado Denver's (UCD) Office of Regulatory Compliance Conflict of Interest and Commitment Management (COIC) program. Persons with a perceived conflict of interest will have such conflicts managed in a way that is appropriate to their participation in the trial. Conflict of Interest management plans are project-specific and are reviewed at least annually. UCD has integrated the institutional conflict of interest management program with its existing program.

15. REFERENCES:

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