ClinicalTrials.gov Identifier: NCT02099864

Oregon Health & Science University
OHSU Knight Cancer Institute
OHSU eIRB Protocol #: 10241

TITLE: ***Molecular Mechanisms Underlying Tumor Progression Despite Enzalutamide Treatment***

Coordinating Center:	The OHSU Knight Cancer Institute Oregon Health & Science University
Participating Sites:	Oregon Health & Science University University of California, San Francisco
Principal Investigator:	Tomasz M. Beer, MD Oregon Health & Science University 3303 SW Bond Ave. Portland, OR 97239 (503) 494-6594 beert@ohsu.edu
OHSU Co-Investigators:	Christopher Ryan, MD Julie Graff, MD Jeremy Cetnar, MD
Collaborators:	Joshi Alumkal, MD Jim Korkola, PhD George V Thomas, MD Laura Heiser, PhD
UCSF Co-Investigator:	Eric Small, MD University of California, San Francisco 415-353-9865 esmall@medicine.ucsf.edu
Statistician:	Yiyi Chen, PhD Oregon Health & Science University 3181 SW Sam Jackson Park Road Portland, OR 97239 503-346-0790 chenyiy@ohsu.edu

Original Protocol Date:	September 23, 2013
Protocol Revision Dates:	September 17, 2014 March 20, 2015 August 29, 2016 January 6, 2017 February 28, 2019 June 25, 2019
Current Version Date:	**March 2, 2020**

SCHEMA

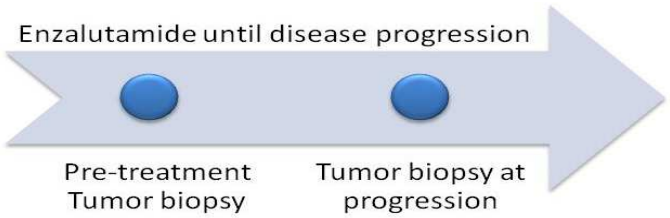


TABLE OF CONTENTS

1.0 OBJECTIVES

1.1 Primary Objective

1.1.1 To assess the correlations between baseline molecular features and pathways and PSA change (</≥ 50% decline) at 12 weeks vs. baseline.

1.2 Secondary Objectives

1.2.1 To measure PSA change at 12 Weeks and at each study visit vs. baseline after enzalutamide treatment.

1.2.2 To measure objective response defined in Section 11.1.1 after enzalutamide treatment.

1.2.3 To assess the correlations between the baseline molecular features and pathways and Progression-Free Survival (defined as time from Day 1 of study drug treatment to date of radiographic progression or clinical progression- See Sec 5.3), Disease-Specific Survival (defined as the time from Day 1 of study drug to date of death from prostate cancer), and Overall Survival (defined as time from Day 1 of study drug treatment to date of death from any cause).

1.2.4 To assess the correlations between the baseline molecular features and pathways and time to PSA progression.

1.2.5 To identify molecular features and cellular pathways present in tumors from men with metastatic CRPC that are progressing despite Enzalutamide treatment.

1.2.6 To explore correlation between baseline molecular features and pathways and changes in Circulating Tumor Cells (CTCs) counts defined in Sec 11.3.1.

1.2.7 To explore correlation between baseline molecular features and pathways and objective response defined in Section 11.1.1.

1.2.8 To assess the correlations between the baseline molecular features and pathways and degree of PSA decline at 12 weeks and maximal PSA decline observed while on study.

1.2.9 To assess the correlations between the baseline molecular features and time on treatment.

1.3 Exploratory Objectives

1.3.1 To assess correlations between cell-free DNA (cfDNA) molecular features from blood and molecular features and pathways from the biopsy samples.

1.3.2 To assess correlations between cfDNA molecular features and endpoints in the primary and secondary objectives listed above.

1.3.3 To assess correlations between cell-free DNA and tumor molecular features and changes in PSA after discontinuing enzalutamide.

1.3.4 To explore correlations with baseline molecular features and tissue histology.

1.3.5 To explore correlations with baseline tissue histology and PSA change, time to PSA progression, time on treatment, progression-free survival, and overall survival.

2.0 BACKGROUND

2.1 Study Disease

Worldwide, prostate cancer ranks third in cancer incidence and sixth in cancer mortality in men. Prostate cancer growth is dependent on androgens, and depleting or blocking androgen action has been a mainstay of treatment for over 6 decades. Hormonal therapies include gonadotropin-releasing hormone (GnRH) analogues, androgen receptor antagonists, ketoconazole, and estrogenic compounds. Tumors that progress despite castrate levels of testosterone in the blood are considered castration-resistant. Despite the early sensitivity of these tumors to hormonal strategies, castration-resistant progression generally represents a transition to the lethal variant of the illness, and most patients ultimately succumb to this disease. The median survival of castration-resistant disease is currently approximately 12 months.[1]

Results of clinical investigations and studies on the molecular profiles of these progressing tumors show that the androgen receptor (AR) remains functional and that the tumors should respond to strategies directed at the androgen receptor signaling axis. Overexpression of the AR has been documented in upwards of 50% of castration-resistant prostate cancer (CRPC) specimens and is believed to contribute to tumor progression.[2] In addition, currently approved AR antagonists have the potential to agonize or stimulate androgen receptor signaling in the setting of AR overexpression, therefore exacerbating or accelerating castration-resistant tumor growth. The decline in serum levels of prostate-specific antigen (PSA) seen upon discontinuation of these agents is consistent with the agonist effects ("anti-androgen withdrawal syndrome").

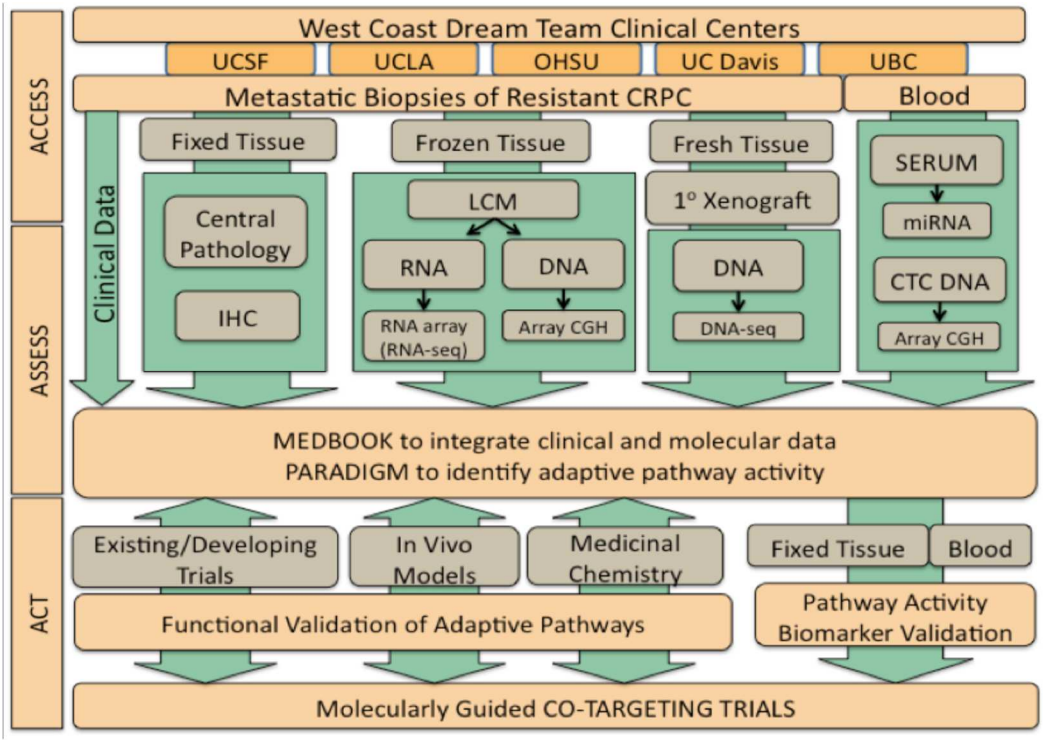
In clinical practice, treatment of advanced prostate cancer is therefore limited by the development of resistance to anti-androgen therapies. Most patients receive two or more hormonal manipulations and are then offered chemotherapy as they continue to progress. A randomized trial in metastatic castration-resistant prostate cancer comparing docetaxel administered every three weeks vs. docetaxel weekly, vs. mitoxantrone has shown a modest survival benefit for docetaxel every 3 weeks, but this response is not durable.[1] Recently, cabazitaxel has been approved for patients who progress on docetaxel on the basis of an open-label Phase 3 study demonstrating a 2.4 month overall survival benefit for men treated with cabazitaxel and prednisone as compared to those treated with mitoxantrone and prednisone.[3] Because many of these resistant tumors continue to overexpress androgen receptors, second generation anti-androgens that are more potent and that are pure antagonists may be effective in patients who have failed docetaxel treatment.

As the central role of the AR in prostate cancer progression became apparent, so did the need for novel strategies to more effectively target the AR. MDV-3100 is the first fruit of this labor and has recently been shown to substantially improve survival in patients with castration resistant prostate cancer (CRPC).[4-6]

This result provides proof of principle for the importance of targeting AR in prostate cancer therapy and produces an urgent need to clarify mechanisms that account for response or eventual resistance to enzalutamide, which are largely unknown. A thorough mechanistic

understanding of the molecular basis for enzalutamide's clinical performance will be an enormous asset in optimizing the use of the drug. Such understanding can yield rationally designed strategies to expand and extend the utility of the drug. Strategies to overcome resistance through novel interventions that interrupt emerging mechanisms of resistance are a particularly compelling long-term outcome. Equally compelling are strategies to extend initial response by optimizing or maximizing those factors that drive response.

The purpose of this study is to determine mechanisms by which CRPC tumors resist treatment with the new anti-androgen enzalutamide.[4,5] This study provides a tremendous opportunity to leverage the molecular analytic resources of a recently funded Stand Up to Cancer (SU2C)/Prostate Cancer Foundation (PCF)/American Association for Cancer Research (AACR) Dream Team grant. This Dream Team intends to deploy a broad array of analytical strategies to comprehensively clarify the molecular basis of treatment resistance in prostate cancer. A cartoon summarizing the major analytic efforts is shown below:



2.2 Study Agent(s)

Enzalutamide is a novel small molecule AR antagonist selected for its activity against prostate cancer cells with overexpressed androgen receptor.[5] Enzalutamide binds more tightly to the AR than does bicalutamide. Unlike bicalutamide, Enzalutamide also inhibits AR function by blocking nuclear translocation of the androgen receptor and deoxyribonucleic acid (DNA) binding. Enzalutamide has no known agonist activity when the androgen receptor is overexpressed. Enzalutamide reduces androgen receptor-dependent PSA release in bicalutamide-resistant prostate cancer cells.

In a mouse xenograft model of castration-resistant prostate cancer using an androgen receptor overexpresssing cell line, Enzalutamide treatment resulted in a dose-dependent reduction in tumor volume ($p < 0.05$ and $p < 0.01$ for mid- and high-dose groups vs.

vehicle, respectively).[5] Enzalutamide treatment decreased tumor volume, resulting in unmeasurable tumors in 1/7 animals in the low-dose group and 3/7 animals in the high-dose group. As expected, bicalutamide had little effect on tumor growth.[5]

In addition to the human AR, the targets for which measurable binding was detected included the human progesterone receptor with a 50% inhibitory concentration (IC50) of 10–25 μM and the rat gamma amino butyric acid-gated chloride channel (IC50 = 2.6 μM; Ki = 2.1 μM [1.0 μg/mL]). Binding of Enzalutamide at 25 μM to the human progesterone receptor was too weak to derive a inhibition constant (Ki) value. No significant binding was detected with the remaining 70 receptors.

The tolerability, pharmacokinetics (PK), and antitumor activity of Enzalutamide were studied in a multi-center, open-label, dose-escalation study of Enzalutamide in 140 patients with castration-resistant prostate cancer.[4] Patients were treated with Enzalutamide at doses of 30–600 mg/day until disease progression or intolerable side effects developed.

The antitumor activity of Enzalutamide was assessed by post-therapy changes in PSA, soft tissue and osseous disease, and circulating tumor cell (CTC) count. PSA declines of ≥ 50% from baseline were observed in 62% of chemotherapy-naïve and 51% of post-chemotherapy patients.[4] At the time of the analyses, the median time to PSA progression was not yet reached for chemotherapy-naïve patients and was 186 days for post-chemotherapy patients.[4]

Among the chemotherapy-naïve patients, there was evidence of radiographic control (no progression) in 80% of patients with evaluable soft tissue disease and 63% of patients with bone lesions.[4] Among the post-chemotherapy patients, there was evidence of radiographic control in 65% of patients with evaluable soft tissue disease and 51% of patients with bone lesions.[4] The median time to radiographic progression was not yet reached for chemotherapy-naïve patients and was 201 days for post-chemotherapy patients.[4] Enumeration of CTCs demonstrated that 91% of patients with favorable pretreatment counts (i.e., < 5 CTCs/7.5 mL of blood) maintained favorable post-treatment counts, while 49% of patients converted from unfavorable pretreatment counts (i.e., ≥ 5 CTCs/7.5 mL of blood) to favorable post-treatment counts.

At the highest dose of 600 mg/day, two of three subjects had dose-limiting toxicities (seizure, rash, respectively).[4] One witnessed seizure at 360 mg/day and a possible seizure at 480 mg/day were also reported. No deaths and no other drug-related serious adverse events were reported. Fatigue was the most frequently reported adverse event, with dose-dependent increases of Grade 3 fatigue (2% at 150, 10% at 240, 21% at 360, and 20% at 480 mg/day groups). The dose of 240 mg/day was defined as the maximum tolerated dose.

Enzalutamide was absorbed rapidly after oral administration, with maximum plasma concentration (Cmax) occurring approximately 30 minutes to 4 hours after dosing. The t1/2 in patients was approximately 1 week (range 3 to 13 days) and did not appear to be affected by the dose size. Enzalutamide plasma concentrations exhibited a low degree of inter- and intra-subject variability and increased linearly with dose. The PK remained linear with time, and there was no evidence of inhibition or autoinduction of metabolism during chronic administration. In accordance with a 1 week t1/2, it took approximately 1 month to reach steady state. The daily fluctuation in steady-state plasma concentrations (i.e., the difference between Cmax and minimum plasma concentration [Cmin]) was low, and PK profiles approximated a constant infusion. At 160 mg/day, mean plasma concentrations fluctuated between 12 μg/mL (Cmin) and 15 μg/mL (Cmax).

2.3 Study and Dose Rationale

Androgen receptor (AR) signaling is the principal molecular signaling driver of prostate cancer progression and dissemination.[2,7] As a consequence, therapies that reduce androgen receptor signaling, principally by reducing systemic ligand production, constitute the mainstay of systemic therapy for advanced prostate cancer.[4,8,9] Both pre-clinical experiments and clinical observations have clarified that resistance to primary hormonal treatments involves androgen-signaling related mechanisms in the majority of patients.[2] A number of such mechanisms have been proposed; they range from somatic receptor mutations of the receptor, receptor splice variants, to a variety of up-stream alterations ranging from persistent endocrine ligand production, to paracrine or even autocrine ligand production, alterations in receptor cofactors, ligand-independent receptor activation, and others.[10-19] As the central role of the AR in prostate cancer progression became apparent, so did the need for novel strategies to more effectively target the AR. MDV-3100 is the first fruit of this labor and has recently been shown to substantially improve survival in patients with castration resistant prostate cancer (CRPC).[4-6]

This result provides proof of principle for the importance of targeting AR in prostate cancer therapy and produces an urgent need to clarify the mechanisms of response and resistance to MDV-3100, which are largely unknown. A thorough mechanistic understanding of the molecular basis for MDV-3100's clinical performance will be an enormous asset in optimizing the use of the drug. Such understanding can yield rationally designed strategies to expand and extend the utility of the drug. Strategies to overcome resistance through novel interventions that interrupt emerging mechanisms of resistance are a particularly compelling long-term outcome. Equally compelling are strategies to extend initial response by optimizing or maximizing those factors that drive response.

For our study, we will treat patients with 160mg PO QD of Enzalutamide. This is the FDA-approved dose used in the recent randomized, placebo-controlled phase III study that demonstrated a five month improvement in overall survival.[6] This dose was well-tolerated.[6]

3.0 STUDY POPULATION

3.1 Inclusion Criteria

3.1.1 Histologically or cytologically confirmed adenocarcinoma of the prostate without pure small cell carcinoma. Patients without histologically confirmed adenocarcinoma may be eligible if both the treating physician and the study PI agree that the patient's history is unambiguously indicative of advanced adenocarcinoma.

3.1.2 Ongoing androgen deprivation therapy with a GnRH analogue or orchiectomy (i.e., surgical or medical castration). Patients who have not had an orchiectomy must maintain effective GnRH-analogue therapy for the duration of the trial.

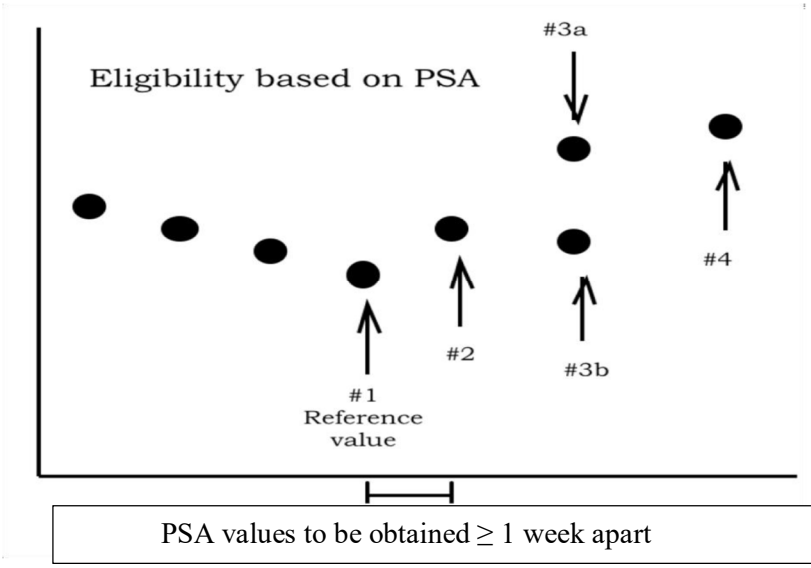
3.1.3 Radiographic evidence of regional or distant metastases with suspected tumor in an area that is safe to biopsy

3.1.4 Willingness to undergo a tumor biopsy at baseline and at disease progression

3.1.5 Serum testosterone level < 50 ng/dL at Screening

3.1.6 Progressive disease by PSA or imaging in the setting of medical or surgical castration. Disease progression for study entry is defined as one or more of the following three criteria:

PSA evidence for progressive prostate cancer which consists of a PSA level of at least 2 ng/ml which has risen on at least 2 successive occasions, at least 1 week apart (#2 & #3a in figure below). If the confirmatory PSA value is less (#3b) than the screening (PSA #2) value, then an additional PSA value (PSA #4) greater than #2 will be required to document progression of ≥ 1 week



- Soft tissue disease progression defined by RECIST 1.1
- Bone disease progression defined by two or more new lesions on bone scan

3.1.7 Patient's physician has already recommended enzalutamide for treatment of progression

3.1.8 ECOG performance status of 0–2

3.1.9 Willing and able to give informed consent

3.1.10 Estimated life expectancy ≥ 6 months

3.1.11 Subjects who have partners of childbearing potential must be willing to use a method of birth control with adequate barrier protection as determined to be acceptable by the principal investigator and sponsor during the study and for 1 week after last study drug administration.

3.1.12 A minimum of 4 weeks elapsed off of anti-androgen therapy prior to enrollment for flutamide and 6 weeks for bicalutamide and nilutamide without evidence of an anti-androgen withdrawal response. Patients who NEVER HAD A PSA decline with the most recent anti-androgen therapy or in whom the response to the most recent anti-androgen was for < 3 months require only a 2 week washout period prior to

first dose of study drug.

3.1.13 A minimum of 4 weeks from prior systemic anti-cancer therapies or 3 weeks for radiation treatment prior to enrollment is required.

3.2 Exclusion Criteria

3.2. 1 Severe, concurrent disease, infection, or co-morbidity that, in the judgment of the investigator, would make the patient inappropriate for enrollment

3.2.2 Previous treatment with docetaxel for metastatic prostate cancer

3.2.3 Known metastases in the brain or active epidural disease (NOTE: patients with treated epidural disease are allowed)

3.2.4 Laboratory Values as follows:

- Absolute neutrophil count < 1,000/μL,
- Platelet count < 75,000/μL,
- Hemoglobin < 9 g/dL at the Screening visit; (NOTE: subject may not have received any growth factors or blood transfusions within seven days of the hematologic laboratory values obtained at the Screening visit).
- Total bilirubin (TBL), alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > 2.5 times the upper limit of normal at the Screening visit.
- Creatinine (Cr) > 2 mg/dL at the Screening visit.
- PT or INR and a PTT > 1.5 times the upper limit of normal

3.2.5 Previous treatment with an agent that blocks adrenal androgen synthesis (e.g.. abiraterone acetate, TAK-700, TOK-001, ketoconazole) or second generation androgen receptor (AR) antagonists (e.g., BMS 641988, ARN-509,TOK-001)

3.2.6 Systemic corticosteroids greater than the equivalent of 10 mg of prednisone per day within 4 weeks of study drug administration are prohibited.

3.2.7 Structurally unstable bone lesions suggesting impending fracture

3.2.8 Previous treatment with Enzalutamide (MDV3100)

3.2.9 Medical contraindications to stopping aspirin, Coumadin or other anticoagulants prior to image-guided tumor biopsies Follow institutional guidelines when determining drugs to avoid and length of washout (OHSU guidelines can be found in Appendix H)

3.2.10 Plans to initiate treatment with an investigational agent during the study

3.2.11 History of seizure or condition that may predispose to seizure. Also, history of loss of consciousness or transient ischemic attack within 12 months of Day 1 visit.

3.2.12 Concomitant use of the strong CYP2C8 inhibitors gemfibrozil or trimethoprim [Bactrim])

3.2.13 History of known malabsorption syndrome or prior surgery(ies) that may lead to malabsorption.

3.2.14 Use of herbal products that may have hormonal anti-prostate cancer activity and/or are known to decrease PSA levels (e.g., saw palmetto) within 4 weeks of study drug administration (Day 1).

3.2.15 Use of the following drugs within 4 weeks of study drug administration:
5 α-reductase inhibitors (finasteride, dutasteride),Estrogens, Cyproterone acetate, biologic, or other agents with anti-tumor activity against prostate cancer, and androgens (testosterone, dihydroepiandrosterone [DHEA], etc.)

3.2.16 A second active malignancy except adequately treated non-melanoma skin cancer or other non-invasive or in situ neoplasm

4.0 REGISTRATION PROCEDURES

4.1 Subject Registration

4.1.1 Local registration

Registration will include the following:
- A completed Subject Enrollment Form
- A completed Eligibility Checklist signed by the investigator
- Complete source documentation for each eligibility criterion
- Signed copies of the most recently IRB-approved, informed consent form and HIPPA authorization

Registrations from all consented subjects must be entered into the Knight Clinical Research Management System (CRMS).

4.1.2 Multicenter Registration
The OHSU coordinating center study team will manage subject registration. Investigators at participating sites will identify eligible subjects and send screening materials with source documents that support eligibility to OHSU in real time and in accordance with study protocol. Designated Knight clinical staff must review and verify eligibility before the participating site may enroll and treat its subject. The OHSU coordinating center team will verify completeness of documents, enter registration information into the Knight CRMS, and assign a study number/identifier. The coordinating center will send an email to the participating site indicating whether or not the subject is eligible, verify registration, and assign a participant number/identifier.

Registration will include the following:
- A completed Subject Enrollment Form
- A completed Eligibility Checklist signed by the investigator
- Complete source documentation for each eligibility criterion
- Signed copies of the most recently IRB-approved, informed consent form and HIPPA authorization

Each site must maintain a screening log of all subjects who sign consent, including screen failures and those who withdraw consent. The log must also document

reason for screen failure.

This log will be submitted to the coordinating center on a regular basis. Participating sites are required to retain, in a confidential manner, sufficient information on each subject so that the subject may be contacted should the need arise.

5.0 TREATMENT PLAN

5.1 Enzalutamide

All patients will receive Enzalutamide 160 mg (four 40 mg capsules) administered orally once daily. Enzalutamide can be taken with or without food. Capsules are to be swallowed whole. Patients will record daily drug administration in a drug diary for tracking purposes (see Appendix F for diary.) Treatment adjustments will be at the discretion of the treating physician and are not a part of this study. See Section 6.0.

5.2 Tumor Biopsy at Study Entry and at Disease Progression

All subjects will undergo a tumor biopsy of a metastatic site at study entry (prior to initiation of Enzalutamide) and after the time of progression. NOTE: Every effort should be made to biopsy NEW lesions if possible. Every effort should be made to perform the progression biopsy prior to discontinuation of Enzalutamide treatment.

The tumor biopsies for this study will be collected and shipped via the established procedures described below from the University of California, San Francisco, Stand Up to Cancer biopsy protocol CC#125519 (OHSU protocol #9204) -Radiologically Guided Biopsies Of Metastatic Castration Resistant Prostate Cancer To Identify Adaptive Mechanisms of Resistance.

All subjects participating in this protocol will also be enrolled in the study referenced above. A separate consent will be obtained for this.

In all subjects, an image-guided (CT or ultrasound) core bone or soft tissue biopsy will be performed (please see Appendix B for more details about this procedure). Patients will be consented to the appropriate procedure prior to biopsy. All screening imaging for patients will be reviewed for eligibility/feasibility of the tumor biopsy. Lesions will be chosen based upon the strength of the evidence suggesting the presence of metastasis and with the goal of minimizing patient risk. New soft tissue or bone lesions of existing lesions with documented radiologic progression should be prioritized for biopsy. If the Radiologist in charge of the procedure cannot identify a lesion amenable for biopsy, the patient will be considered a screening failure.

The biopsies will be performed in an interventional radiology suite with radiological guidance (typically CT or MRI) in accordance with the standard operating procedure in **Appendix B** and institutional standards. CT or MRI will confirm designated lesions immediately prior to biopsy. Once the target lesion(s) identified, six (6) to eight (8) biopsies will be performed. Preferably, a 16 gauge Bonopty^{TM} needle or biopsy needle with an equivalent 16g bore will be used to biopsy the metastatic lesion. If the lesion is a bone metastasis, the Bonopty needle will be passed through the cortical bone and into the target lesion. Optimal results are obtained when the biopsies are performed on medulary bone directly adjacent to blastic lesion. Soft tissue biopsies should be taken so that a core of

approximately 10 to 20 mm in length is obtained. Core biopsies will be extracted: 2 will be placed in neutral-buffered formalin and 2 to 4 will be immediately frozen on a pre-frozen bed of OCT (Optimal Cutting Temperature compound used for frozen sections), covered with additional OCT, and kept on dry ice or at -80° C (see Appendix B).

Please see **Appendix B** for the handling of these tissues.

5.3 Definition of Progression for Biopsy upon Progression

Radiographic progression:

a) Soft tissue progression: by RECIST v1.1
   i. Progression at the first scheduled reassessment at Week 12 by RECIST 1.1 must be confirmed by a second scan performed 6 or more weeks later. Confirmatory scans should show progressively worsening disease compared to the Week 12 scan.
   ii. Progressive soft tissue disease on CT or MRI per RECIST 1.1 seen for the first time after Week 12 does not require confirmation.

and/or

b) Bone scan progression: The appearance of $\geq$ 2 new lesions that are confirmed.
   i. New lesions at the first scheduled reassessment at Week 12 must be confirmed by a second bone scan performed 6 or more weeks later. The confirmatory bone scan should show $\geq$ 2 additional new lesions compared to the Week 12 scans.
   ii. New lesions compared to week 12 that are detected for the first time after the Week 12 reassessment should be confirmed by a second assessment performed 6 or more weeks later. Confirmatory scans should show $\geq$ 2 new lesions compared to the prior reassessment.

c) If the investigator's clinical assessment based on patient symptoms, laboratory data, and radiographs suggests the patient may still be clinically benefitting the patient may continue on treatment beyond protocol defined radiographic progression.. Every attempt should be made to obtain a biopsy when the decision is made to discontinue Enzalutamide.

Clinical progression

d) Clinical progression at investigator's discretion. If clinical progression is the only trigger for biopsy, tumor assessment by CT of the chest/abdomen/pelvis and Bone scan should be obtained prior to biopsy. (The CT and Bone scan does not need to be repeated if scans were done within 30 days.)

   Note that PSA progression alone does not meet criteria for the progression-triggered biopsy. However, if the investigator plans to change therapy due to PSA progression a biopsy may be obtained.

   Every attempt should be made to obtain the progression biopsy. If in the opinion of the investigator the subject's progression biopsy cannot be performed the reason will be documented and the subject will continue to be followed per protocol.

5.4 General Concomitant Medication and Supportive Care Guidelines

Patients should receive supportive care. This includes antibiotics, anti-emetics, pain

medications, and bone targeted therapy. Growth factors (GCSF or Erythropoietin) or transfusion with blood products are allowed as long as these cytopenias are not felt to be related to Enzalutamide. **Please also see section 8.2.**

5.5 Banking of Specimens for Potential Future Research

Specimens collected and any serum/plasma will be banked for future research under the "Master Protocol for Cancer Research Specimen Bank and Database" (OHSU IRB 2816), and will be used to search for biomarkers of response and resistance to therapy. Future research may include genetic research.

5.6 Duration of Treatment

Subjects will remain on Enzalutamide treatment until after progression-triggered biopsy, unacceptable toxicity (including any seizures), any adverse event that is intolerable to the subject and cannot be ameliorated by the use of adequate medical intervention or that in the opinion of the physician would lead to undue risk to the subject if dosing continued, withdrawal of consent or if the physician feels it is in the best interest of the subject to discontinue therapy.

5.7 Duration of Follow-Up

Subjects who have an adverse event(s) related to study treatment (biopsy or Enzalutamide) will be followed until resolution or stabilization of the adverse event. Subjects who discontinue Enzalutamide treatment prior to confirmed radiographic progression will be followed for progression free survival and overall survival. All subjects will be followed for overall survival. See Section 10.3

6.0 DOSING DELAYS/DOSE MODIFICATIONS

Dose Delays

Subjects requiring > 28 consecutive days of drug interruption will meet criteria for study treatment discontinuation.

Dose Modifications

If a patient experiences a seizure, permanently discontinue study drug treatment.

For < Grade 3 AEs that are not seizures that are probably or definitely related to enzalutamide, a dose reduction to 120mg is allowed. A further dose reduction to 80mg for < Grade 3 AEs that are not seizures but that are persistent despite 120mg dose reduction is allowed.If a patient experiences a ≥ Grade 3AE that is not a seizure and is probably or definitely related to ***study treatment***, withhold dosing until symptoms improve to Grade 1 or baseline. Once the adverse event resolves to Grade 1 or baseline, the study drug should be dose reduced to 120mg if treatment is resumed. A further dose reduction to 80mg is allowed if symptoms recur.

7.0 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

7.1 Adverse Events and Potential Risks List(s)

Enzalutamide

The most common adverse drug reactions (≥ 5%) reported in patients receiving Enzalutamide in the randomized clinical trial 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. Grade 3 and higher adverse reactions were reported among 47% of Enzalutamide-treated patients and 53% of placebo-treated patients. Discontinuations due to adverse events were reported for 16% of Enzalutamide-treated patients and 18% of placebo-treated patients. The most common adverse reaction leading to treatment discontinuation was seizure, which occurred in 0.9% of the Enzalutamide-treated patients compared to none (0%) of the placebo-treated patients.

Table below shows adverse reactions reported in the randomized clinical trial that occurred at a ≥ 2% absolute increase in frequency in the Enzalutamide arm compared to the placebo arm.

	XTANDI N = 800		Placebo N = 399	
	Grade 1-4 (%)	Grade 3-4 (%)	Grade 1-4 (%)	Grade 3-4 (%)
General Disorders				
Asthenic Conditions[a]	50.6	9.0	44.4	9.3
Peripheral Edema	15.4	1.0	13.3	0.8
Musculoskeletal And Connective Tissue Disorders				
Back Pain	26.4	5.3	24.3	4.0
Arthralgia	20.5	2.5	17.3	1.8
Musculoskeletal Pain	15.0	1.3	11.5	0.3
Muscular Weakness	9.8	1.5	6.8	1.8
Musculoskeletal Stiffness	2.6	0.3	0.3	0.0
Gastrointestinal Disorders				
Diarrhea	21.8	1.1	17.5	0.3
Vascular Disorders				
Hot Flush	20.3	0.0	10.3	0.0
Hypertension	6.4	2.1	2.8	1.3
Nervous System Disorders				
Headache	12.1	0.9	5.5	0.0
Dizziness[b]	9.5	0.5	7.5	0.5
Spinal Cord Compression and Cauda Equina Syndrome	7.4	6.6	4.5	3.8
Parasthesia	6.6	0.0	4.5	0.0
Mental Impairment Disorders[c]	4.3	0.3	1.8	0.0
Hypoesthesia	4.0	0.3	1.8	0.0
Infections and Infestations				
Upper Respiratory Tract Infection[d]	10.9	0.0	6.5	0.3
Lower Respiratory Tract And Lung Infection[e]	8.5	2.4	4.8	1.3
Psychiatric Disorders				
Insomnia	8.8	0.0	6.0	0.5
Anxiety	6.5	0.3	4.0	0.0
Renal And Urinary Disorders				
Hematuria	6.9	1.8	4.5	1.0
Pollakiuria	4.8	0.0	2.5	0.0
Injury, Poisoning And Procedural Complications				
Fall	4.6	0.3	1.3	0.0
Non-pathologic Fractures	4.0	1.4	0.8	0.3
Skin and Subcutaneous Tissue Disorders				
Pruritus	3.8	0.0	1.3	0.0
Dry Skin	3.5	0.0	1.3	0.0
Respiratory Disorders				
Epistaxis	3.3	0.1	1.3	0.3

[a] Includes asthenia and fatigue.
[b] Includes dizziness and vertigo.
[c] Includes amnesia, memory impairment, cognitive disorder, and disturbance in attention.
[d] Includes nasopharyngitis, upper respiratory tract infection, sinusitis, rhinitis, pharyngitis, and laryngitis.
[e] Includes pneumonia, lower respiratory tract infection, bronchitis, and lung infection.

Seizure

In the randomized clinical trial, 7 of 800 (0.9%) patients treated with Enzalutamide 160 mg once daily experienced a seizure. No seizures occurred in patients treated with placebo. Seizures occurred from 31 to 603 days after initiation of Enzalutamide. Patients experiencing seizure were permanently discontinued from therapy and all seizures resolved. There is no clinical trial experience re-administering Enzalutamide to patients who experienced seizures.

The safety of Enzalutamide in patients with predisposing factors for seizure is not known because these patients were excluded from the trial. These exclusion criteria included a history of seizure, underlying brain injury with loss of consciousness, transient ischemic attack within the past 12 months, cerebral vascular accident, brain metastases, brain arteriovenous malformation or the use of concomitant medications that may lower the seizure threshold.

Because of the risk of seizure associated with Enzalutamide use, patients should be advised of the risk of engaging in any activity where sudden loss of consciousness could cause serious harm to themselves or others.

Risks of Biopsy include: bleeding, pain, damage to adjacent organs, and infection.

7.2 Adverse Event Characteristics

Adverse events will be collected and attribution will be designated for any untoward clinical experience, including but not limited to adverse events related to biopsies and associated sedation, venipuncture, as well as Enzalutamide

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting, with the exception of lab abnormalities which will be managed as described below. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

Attribution of the AE: Unrelated, unlikely, possibly, probably, definitely

Laboratory Abnormalities

An abnormal lab result should be reported as an adverse event and graded per CTCAE if the test result is deemed Clinically Significant (CS) by the responsible or treating physician. Clinical significance is based on clinical judgement and individual patient situation, at the discretion of the physician. Examples of a test result that may be considered CS are described below. This list is not exhaustive and a result that is similar to an example below may not be considered CS if the treating physician determines it to be Not Clinically Significant (NCS):

- Test result is associated with accompanying symptoms, and/or
- Test result requires additional diagnostic testing or medical/surgical intervention, and/or
- Test result leads to a change in study dosing outside of protocol-stipulated dose adjustments or discontinuation from the study, significant additional concomitant drug treatment, or therapy, and/or
- Test result is considered to be an adverse event by the Investigator

7.3 OHSU IRB Reporting of Unanticipated Problems and Adverse Events

Unanticipated Problems (UP) and Adverse Events (AE) will be reported to OHSU IRB according to the policies, procedures and guidelines posted on the OHSU IRB web site http://www.ohsu.edu/research/rda/irb/policies.shtml.

Fatal and life-threatening events must be reported to OHSU IRB within 7 calendar days after the PI learns of the event. If any of these require a change (as determined by the PI or the IRB) to the protocol or consent form, the PI will make those changes promptly and submit the revised documents to the OHSU IRB.

All other UP reports will be submitted to OHSU IRB no later than 15 calendar days of notification of the event. If the event requires changes as determined by the PI or the IRB) to the protocol or consent form, the PI will make the changes promptly and submit the revised documents to the IRB. UP and AE reports are submitted through OHSU e-IRB and will be reviewed by OHSU IRB.

7.4 Central Reporting of Adverse Events for Multicenter Studies

The SAE/UP reporting for multicenter investigator initiated clinical trials will follow the guidelines outlined in the OHSU Knight Cancer Institute Multi-Center Investigator Initiated Trials Coordinating Center Operations Manual.

A participating site must report an SAE to the to the institution's local IRB for action as required, as well as to the OHSU coordinating center study team by fax or email within 24 hours of learning of the event.

The OHSU coordinating center study team will review and submit SAEs to the FDA, OHSU IRB, and any other required contacts as required by the Knight Data Safety Monitoring Plan. The principal investigator at the Coordinating Center is responsible for distributing IND and/or IDE Action Letters or Safety Reports, as applicable, to participating institutions for review and submission to their institution's local IRB.

7.5 MedWatch/SAE Reporting

An AE is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Results in death,
- Is life threatening (an AE is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.),
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions,
- Results in congenital anomaly, or birth defect,
- Requires inpatient hospitalization or leads to prolongation of hospitalization (hospitalization for treatment/observation/examination caused by AE is to be considered as serious),
- Other medically important events.

Within 24 hours of awareness of a serious adverse event, whether or not related to the study drug, the Investigator will complete and submit a Medwatch 3500A Form to FDA,

containing all required information (reference 21 CFR 312.32). The Investigator will submit a copy of this MedWatch 3500A form to Astellas by either e-mail or fax, within the same timeframe.. If submission of this SAE to FDA or Astellas or is not possible within 24 hours, the Investigator's local drug safety contact (IRB, etc.) should be informed by phone.

The SAE documentation, including the Medwatch 3500A Form and available source records should be emailed or faxed to (See Appendix D for the fax cover sheet):

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

8.0 PHARMACEUTICAL INFORMATION

8.1 Enzalutamide

Product description: Enzalutamide (MDV3100) will be prescribed at 160mg per day. Enzalutamide will be provided as liquid-filled soft gelatin capsules for oral administration. Each capsule contains 40 mg of Enzalutamide as a solution in caprylocaproyl polyoxylglycerides.

Route of administration: Oral, daily.

8.1.1 Laboratory Abnormalities

In the randomized clinical trial, Grade 1-4 neutropenia occurred in 15% of patients on Enzalutamide (1% Grade 3-4) and in 6% of patients on placebo (no Grade 3-4). The incidence of Grade 1-4 thrombocytopenia was similar in both arms; 0.5% of patients on Enzalutamide and 1% on placebo experienced Grade 3-4 thrombocytopenia. Grade 1-4 elevations in ALT occurred in 10% of patients on Enzalutamide (0.3% Grade 3-4) and 18% of patients on placebo (0.5% Grade 3-4). Grade 1-4 elevations in bilirubin occurred in 3% of patients on Enzalutamide and 2% of patients on placebo.

8.1.2 Infections

In the randomized clinical trial, 1.0% of patients treated with Enzalutamide compared to 0.3% of patients on placebo died from infections or sepsis. Infection-related serious adverse events were reported in approximately 6% of the patients on both treatment arms.

8.1.3 Falls and Fall-related Injuries

In the randomized clinical trial, falls or injuries related to falls occurred in 4.6% of patients treated with Enzalutamide compared to 1.3% of patients on placebo. Falls were not associated with loss of consciousness or seizure. Fall-related injuries were more severe in patients treated with Enzalutamide and included non-pathologic fractures, joint injuries, and hematomas.

8.1.4 Hallucinations

In the randomized clinical trial, 1.6% of patients treated with Enzalutamide were reported to have Grade 1 or 2 hallucinations compared to 0.3% of patients on placebo. Of the patients with hallucinations, the majority were on opioid-containing medications at the time of the event. Hallucinations were visual, tactile, or undefined.

8.1.5 Pregnancy

Enzalutamide can cause fetal harm when administered to a pregnant woman based on its mechanism of action. While there are no human or animal data on the use of Enzalutamide in pregnancy and Enzalutamide is not indicated for use in women, it is important to know that maternal use of an androgen receptor inhibitor could affect development of the fetus. Enzalutamide is contraindicated in women who are or may become pregnant while receiving the drug. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, apprise the patient of the potential hazard to the fetus and the potential risk for pregnancy loss. Advise females of reproductive potential to avoid becoming pregnant during treatment with Enzalutamide.

It is recommended that women who are pregnant or who may be pregnant should wear gloves if they need to touch or handle Enzalutamide capsules.

8.2 Prior and Concomitant Therapy

8.2.1 Drugs that Inhibit or Induce CYP2C8

Concomitant use of the strong CYP2C8 inhibitors gemfibrozil or trimethoprim [Bactrim]) is excluded in this protocol.

8.2.2 Drugs that Inhibit or Induce CYP Enzymes

APPENDIX E provides a list of potent CYP enzyme inhibitors and inducers that may have a theoretical concern of potential drug-drug interactions with MDV3100. In vitro drug metabolism studies suggest that MDV3100 may have the potential to induce CYP3A4 and to inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5; therefore, concomitant medications that are substrates of any of these enzymes should be used with caution, and relevant monitoring should be considered, especially for substrates known to cause seizure, because the possibility of drug-drug interactions cannot be fully excluded. Since the metabolism of MDV3100 is not known, caution should be taken for the concomitant use of strong inhibitors and inducers of CYP enzymes and alternative products used when available.

Co-administration of a strong CYP3A4 inhibitor (itraconazole) increased the composite AUC of enzalutamide plus Ndesmethyl enzalutamide by 1.3 fold in healthy volunteers

The effects of CYP3A4 inducers on the pharmacokinetics of enzalutamide have not been evaluated *in vivo*. Co-administration of Enzalutamide with strong CYP3A4 inducers (e.g., 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 (e.g.,

bosentan, efavirenz, etravirine, modafinil, nafcillin) and St. John's Wort may also reduce the plasma exposure of Enzalutamide and should be avoided if possible

8.2.3 Effect of Enzalutamide on Drug Metabolizing Enzymes

Enzalutamide is a strong CYP3A4 inducer and a moderate CYP2C9 and CYP2C19 inducer in humans. At steady state, Enzalutamide reduced the plasma exposure to midazolam (CYP3A4 substrate), warfarin (CYP2C9 substrate), and omeprazole (CYP2C19 substrate). Concomitant use of Enzalutamide with narrow therapeutic index drugs that are metabolized by CYP3A4 (e.g., alfentanil, cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus and tacrolimus), CYP2C9 (e.g., phenytoin, warfarin) and CYP2C19 (e.g., S-mephenytoin) should be avoided, as Enzalutamide may decrease their exposure. If co-administration with warfarin cannot be avoided, conduct additional INR monitoring.

8.2.4 Effect of Other drugs on Enzalutamide

In a drug-drug interaction trial in healthy volunteers, a single 160 mg oral dose of Enzalutamide was administered alone or after multiple oral doses of gemfibrozil (strong CYP2C8 inhibitor). Gemfibrozil increased the AUC0-inf of Enzalutamide plus N-desmethyl enzalutamide by 2.2-fold with minimal effect on Cmax. The results are summarized in Figure 1.

NOTE: Gemfibrozil and trimethoprim (Bactrim) are prohibited during study treatment.

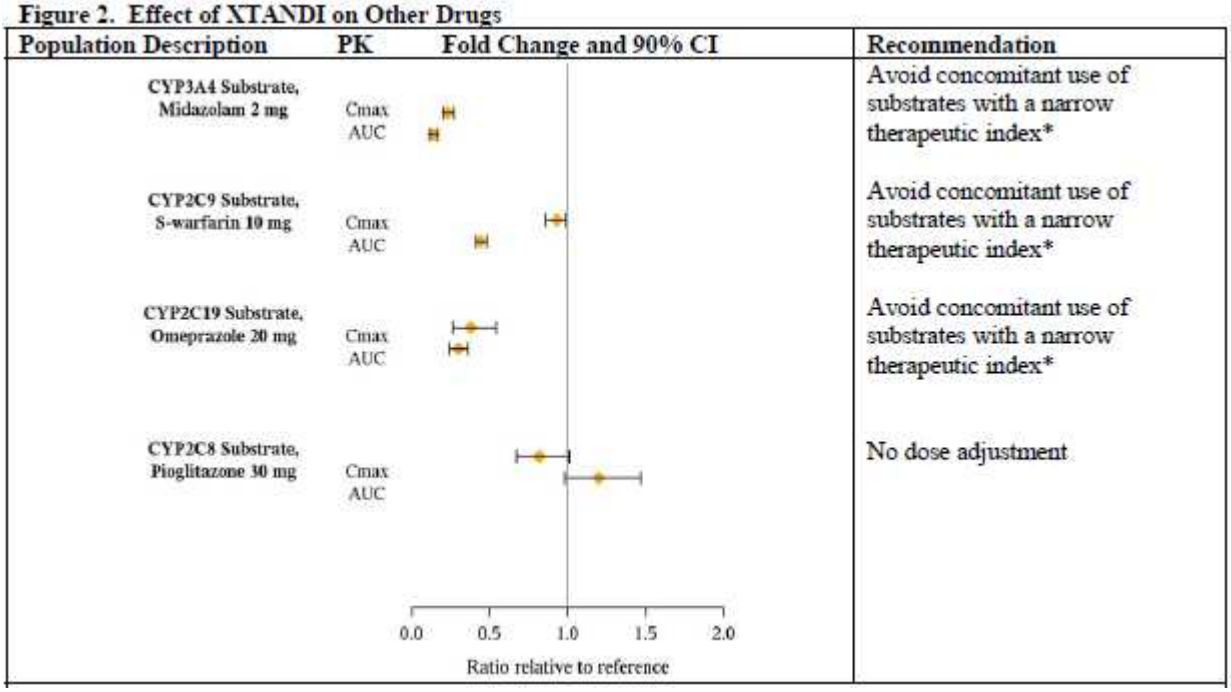
In a drug-drug interaction trial in healthy volunteers, a single 160 mg oral dose of Enzalutamide was administered alone or after multiple oral doses of itraconazole (strong CYP3A4 inhibitor). Itraconazole increased the AUC0-inf of Enzalutamide plus N-desmethyl enzalutamide by 1.3-fold with no effect on Cmax. The results are summarized in Figure 1.

The effects of CYP2C8 and CYP3A4 inducers on the exposure of Enzalutamide have not been evaluated *in vivo*.

8.2.5 Effect of Enzalutamide on Other Drugs

In an *in vivo* phenotypic cocktail drug-drug interaction trial in patients with castration-resistant prostate cancer, a single oral dose of the CYP probe substrate cocktail (for CYP2C8, CYP2C9, CYP2C19, and CYP3A4) was administered before and concomitantly with Enzalutamide (following at least 55 days of dosing at 160 mg daily). The results are summarized in Figure 2. Results showed that *in vivo*, at steady state, Enzalutamide is a strong CYP3A4 inducer and a moderate CYP2C9 and CYP2C19 inducer. Enzalutamide did not cause clinically meaningful changes in exposure to the CYP2C8 substrate.

Figure 2. Effect of XTANDI on Other Drugs



In vitro, enzalutamide, N-desmethyl enzalutamide, and the major inactive carboxylic acid metabolite caused direct inhibition of multiple CYP enzymes including CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5; however, subsequent clinical data showed that Enzalutamide is an inducer of CYP2C9, CYP2C19, and CYP3A4 and had no clinically meaningful effect on CYP2C8 (see Figure 2). *In vitro*, enzalutamide caused time-dependent inhibition of CYP1A2.

In vitro studies showed that enzalutamide caused induction of CYP3A4 and that enzalutamide is not expected to induce CYP1A2 at therapeutically relevant concentrations. *In vitro*, enzalutamide, N-desmethyl enzalutamide, and the major inactive carboxylic acid metabolite are not substrates for human P-glycoprotein. *In vitro*, enzalutamide and N-desmethyl enzalutamide are inhibitors of human P-glycoprotein, while the major inactive carboxylic acid metabolite is not.

8.2.6 The following medications are prohibited within 4 weeks of study drug administration (Day 1):

Flutamide (Patients who never had a PSA decline with the most recent anti-androgen therapy or in whom response to the most recent anti-androgen was for <3 months require only a 2 week washout.)

Bicalutamide or nilutamide (6 weeks washout required for these two agents. Patients who never had a PSA decline with the most recent anti-androgen therapy or in whom response to the most recent anti-androgen was for <3 months require only a 2 week washout.)

5 α-reductase inhibitors (finasteride, dutasteride)

Estrogens

Cyproterone acetate

Biologic, or other agents with anti-tumor activity against prostate cancer

Systemic glucocorticoids greater than the equivalent of 10 mg per day of prednisone

Herbal medications that have known hormonal anti-prostate cancer activity and/or are known to decrease PSA levels (i.e., saw palmetto)

Androgens (testosterone, dihydroepiandrosterone [DHEA], etc.)

8.2.7 Medications that may lower the seizure threshold should be used with caution

APPENDIX G provides a partial list of medications that may lower the seizure threshold.

9.0 BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

Gene expression analysis

Biopsy Samples

Radiology-guided biopsies will be handled and processed by the study investigators and their assigned staff. Biopsies will be collected in the radiology suite. As biopsies are performed, two will be placed in 10% NB formalin solution for eventual decalcification (if required) and paraffin embedding and four to six biopsies will be placed immediately on pre-frozen pallets of OCT. The placement of biopsies on previously frozen OCT rapidly freezes the tissue and immobilizes the biopsy.

The frozen sample will be labeled with the patient's unique, coded number and taken directly or shipped by next day Air on dry ice to the laboratory of Dr. Phillip Febbo at UCSF (See **Appendix B** for shipping details).

Samples have to be shipped Monday through Thursday, and Saturday delivery is not allowed. Avoid shipping the Thursday prior to a UCSF holiday without prior approval by the study chair.

Frozen tumor biopsies will be cryosectioned and a slide stained with Hematoxylin and Eosin (H&E) for histological evaluation. If cancer is present, additional slides will be made and tissue subjected to laser capture microdissection for RNA and DNA isolation.

The two biopsies placed in NB formalin will be shipped to the Thomas laboratory at OHSU for processing and further work up (**Appendix B** for shipping details). Paraffin embedded biopsies will undergo surface decal (using Immunocal, from Decal Chemical Corp). Hematoxylin and Eosin (H&E) staining will be performed to confirm the presence of prostate cancer and document the extent of involvement. FFPE samples will be paraffin-embedded and stored at 4° C in airtight containers to minimize oxidation of proteins within the blocks.

Unstained sections will be cut and subsequently used for immunohistochemical staining. DNA will be extracted from the FFPE tissues (from unstained slides or tissue cores or LCM depending on the

amount to tumor present) for downstream sequencing on the Ion Torrent panel. We anticipate that 240 of the 300 samples (80%) will demonstrate histologically identified prostate cancer.

The H&E stained slides for both the frozen and the paraffin-embedded biopsies will be sent to the Huang lab at UCLA for digital scanning and central pathological review to determine the presence or absence of tumor and the approximate percentage of tumor in each biopsy.

Determination of AR Activity

Preparation of RNA and DNA

Frozen biopsies will be processed for RNA analysis using laser capture microdissection and RNA amplification using adaptations of previously published methods [20]. Briefly, 8 micro frozen sections are obtained from each biopsy specimen and stained with H&E. Sections will be loaded into the Aperio Digital Imaging system and reviewed by the Dream Team Molecular Pathologists (Drs. Thomas and Huang) and Dr. Febbo to establish the presence or absence of prostate cancer and identify the biopsies most likely to provide sufficient tumor for RNA analysis. Multiple sequential 8 micron biopsies are performed prior to rapid H&E staining and dehydration with sequential immersion in xylene. The air-dried slides are put into a LCM system and prostate cancer cells are identified and collected. Cell material selected for analysis is dissolved in RNA or DNA isolation buffer and RNA and DNA are isolated separately using standard commercially available kits. RNA and DNA are quantified using the RiboGreen or PicoGreen kits (Invitrogen, Inc) and RNA quality is assessed with an Agilent 2100 Bioanalyzer using the RNA 6000 Pico Kit (Agilent, Inc). Samples with sufficient RNA and RNA with clear 18S and 28S bands present on the bioanalyzer are amplified and labeled with biotin using the NuGen WT Ovation kit (NuGen Inc). Amplified and labeled target is again quantified and 2.5 micrograms of product are sent to Expression Analysis, Inc for hybridization to Affymetrix U133 Plus 2.0 arrays. Similarly, DNA is collected from separate but adjacent frozen sections and isolated. DNA is quantified using PicoGreen quantification.

For RNA analysis to progress and to apply the AR signature, quality control parameters have been set for RNA and microarray data. RNA samples will be considered of sufficient quality or quantity if they have un-detectable 18S and 28S peaks on the Agilent Bioanalyzer **or** a RIN < 3 AND sufficient RNA to detect an 18S peak. If RNA quantity is below that assessable by the Agilent Bioanalyzer or of questionable quality but sufficient for RNA amplification based upon the RiboGreen results, the specimen will be processed for microarray analysis and quality assessment of the microarray data will be used to determine technical success. Samples resulting in insufficient probe creation (an insufficient amount for hybridization) following RNA amplification with NuGen WT Ovation, will be re-extracted once and RNA amplification repeated. If two RNA amplifications failed, the sample will be considered a technical failure. Once samples are hybridized, array-based exclusion criteria include standard QC measures for Affymetrix platforms (e.g. %present, 3'/5' ratios), and outliers in relation to the normalization dataset, as determined by principal components analysis (PCA).

Application of the AR Signature

The predictor has been derived from the LNCaP cell line data using established methodologies [21]. Briefly, prior to statistical modeling, LNCaP cell line gene expression data is filtered to exclude probe sets with minimal variation (lowest 10% of genes based upon standard deviation of expression across the 10 samples used to create signature).

When predicting the AR activity of tumor samples, gene selection and identification is based on the training data from the LNCaP cell line, and then metagene values are computed using the principal components of the expression data. Bayesian fitting of binary probit regression models to the training data then permits an assessment of the relevance of the metagene signatures in within-sample classification [22], and estimation and uncertainty assessments for the binary regression weights mapping metagenes to probabilities. Given the LNCaP training set of expression vectors (of values across metagenes) representing two biological states (AR active and or inactive), a binary probit regression model, of predictive probabilities for each of the two states (high vs. low) for each case is estimated using Bayesian methods [21,22] producing estimated relative probabilities – and associated measures of uncertainty – of AR activity across the prostate cancer samples.

Determination of Adaptive Pathway Activity

Adaptive pathway activity will be determined using both immunohistochemistry and integrative pathway analysis with PARADIGM. Histological sections from the fixed, paraffin-embedded biopsies containing tumor will be used for ICH. RNA and DNA from the image-guided biopsies will be processed for RNA-sequencing and array comparative genome hybridization (aCGH) which will be used by PARADIGM to determine pathway activity.

Immunohistochemistry for selected Adaptive Pathways

Four immunohistochemcial markers will be performed to determine the activity of select adaptive pathways including: (1) nuclear AR staining [23\24]; (2) PTEN protein [25,26]; (3) phosphorylated hsp27[27]; and (4) N-cadherin [28]. IHC quantification will be performed with automated digital image analysis algorithms to rigorously and systematically measure staining intensities, using the inFORM image analysis software (CRi/Caliper Life Sciences, Hopkinton, MA) [29]. Established qualitative assessments will only be used if staining characteristics preclude automated image analysis, and will be performed by both pathologists while masked to associated clinical or molecular data.

aCGH Analysis

aCGH analysis will be performed on tumor biopsies and CTCs. Briefly, 0.5 ug of each genomic DNA will be fluorescently labeled by following the NimbleGen enzymatic labeling protocol, which employs Cy3 and Cy5 labeled random nanomers (TriLink Biotechnologies), a heat fragmentation step at 98° C for 10 minutes, and amplification with Klenow fragment 5'-3'exo- (NEB). Five micrograms of each Cy5-labeled sample will be co-hybridized with 5 ug of Cy3-labeled human male reference DNA (Promega) on Agilent SurePrint G3 Human Catalog CGH 4x180K (Part No. G4449A) following the hybridization and washing conditions from the Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis Protocol v6.2. Arrays will be scanned with the Agilent DNA Microarray Scanner, and quantified with Feature Extraction 10.5.1.1. CGH processed signal will then be uploaded into Biodiscovery Nexus software, where the quality will be assessed and data visualized and analyzed for copy number variation to be used for pathway analysis and to determine if tumor biopsies and CTCs in the same patient share genetic events.

RNA-sequencing

We will apply a newly developed strategy to perform RNA sequencing from minute amounts of total RNA [30]. LCM collected material will be lysed with 5 µl Prelude Lysis Buffer (NuGen, San Carlos, CA); subjected to cDNA synthesis and amplification without

transfer (NuGen, Ovation RNA-seq with some modifications to the standard protocol); selected for size and processed using the mixed cDNA standard library preparation performed using TruSeq protocol (Illumina, San Diego, CA). Paired-end sequencing will be performed on the Illumina HiSeq 2000. RNA-seq is generating very high quality data and may replace RNA microarrays for the determination of AR signature activity.

Tumor DNA-sequencing

We will use next generation sequencing to detect potentially actionable mutations with patient specimens as well as perform whole exome sequencing to identify activating mutations and other genetic events. Genomic DNA from individuals will be extracted from collected blood samples (germ line) and tumor biopsies; sonicated to approximately 50 - 200 bp fragments; and used to make a library for paired-end sequencing (Illumina) or for sequencing within the OHSU CLIA laboratory (Ion Torrent sequencing). using established protocols [31].

We will use whole genome sequencing or Sanger sequencing of cfDNA from blood and DNA from the tumor biopsy specimens. This will enable us to identify molecular features (mutations, including but not limited to the AR, copy number alterations, and gene fusions) that may predict resistance or early progression despite enzalutamide treatment.

As RNA- and DNA-sequencing can provide similar molecular information as RNA microarrays and aCGH, respectively, it is our expectation that sequence-based analysis will likely displace array-based analysis during the study. The molecular platform deemed to provide the best data for pathway analysis (AR and adaptive pathways) will be used by investigators.

Integrative Pathway Analysis to Associate Adaptive Pathways

Pathway analysis will be both hypotheses driven based upon previously identified adaptive pathways as well as unbiased (using pathway analyses to associate biological mechanisms to drug resistance). RNA-seq and aCGH data (as well as any additional molecular analyses) will be imported into MedBook. Using the molecular data generated from patient samples, we will use a software program called PARADIGM for integrative pathway-based analysis.

Adaptive Pathway Activity Analysis

PARADIGM analysis is well suited to the detection of significant alteration in adaptive pathways, and additional key known cancer pathways. The current SuperPathway database contains over 16,000 features covering 5,500 proteins (roughly 25% of the genome), which are heavily cancer-oriented since the construction of the database was built from NCI's Pathway Interaction Database (PID), Reactome, and BioCarta. Future additions will come from KEGG and WikiPathways. Using clinical and molecular data in MedBook, our team will apply PARADIGM analysis to determine which adaptive pathways are relatively active in each individual biopsied.

Unbiased Pathway Analysis

In order to identify novel mechanisms underlying eventual disease progression or resistance, we will expand our pathway database to include predicted interactions by mining functional genomics resources. We have developed the Differential Pathway Signature Analysis (DPS) to integrate pathway analysis with clinical response or gene mutation. The DPS method is built on many tools: PARADIGM [32], Significance Analysis of Microarrays (SAM) [33], Cluster [34], and Java Treeview. PARADIGM is used to integrate gene expression and DNA-based events (copy number variation, fusion, mutation etc) into

a combined pathway score. These scores are called IPLs (inferred pathway levels). PARADIGM inferred pathway features are preferable over gene expression since PARADIGM integrates knowledge from both RNA-seq and aCGH.

PARADIGMs output, a relative measure of pathway activities within and across samples as continuous variables, will be used to determine the distribution of pathway activity and the association with clinical features.

10.0 STUDY PROCEDURES AND SCHEDULE OF EVENTS

10.1 Screening/ Visit

- Medical history
- ECOG performance status
- A complete physical exam including vitals (blood pressure, heart rate, respiration rate, temperature and weight)
- Laboratory assessments (hematology, chemistry, testosterone, PSA, PT/INR, APTT and LDH
- Scans (CT scan chest/abdomen/pelvis (preferably with contrast) or MRI (if CT scan is contraindicated) and bone scan)

Hematology, PT/INR, and APTT are to be conducted no more than 14 days prior to biopsy. The remaining screening evaluations are to be conducted within 35 days of enrollment (day of pre-treatment biopsy). Biopsy must be no more than10 days before starting Enzalutamide treatment.

10.2 Study Visits

10.2.1 Enrollment / Baseline biopsy:

- After eligibility is confirmed and all screening procedures complete per 10.1, subject will be registered for the study according to registration procedures outlined in Section 4.0.
- All eligible subjects will undergo a biopsy of a metastatic lesion as described in Section 5.2 and Appendices B and C.

10.2.2 Day 1 (start daily dosing of Enzalutamide) pre-dose:

- ECOG performance status
- A complete physical exam including vitals
- Laboratory assessments (hematology, chemistry, and PSA)

10.2.3 Week 6:

- ECOG performance status
- A complete physical exam including vitals
- Laboratory assessments (hematology, chemistry, and PSA)

10.2.4 Week 12:

- ECOG performance status
- A complete physical exam including vitals
- Laboratory assessments (hematology, chemistry, and PSA)
- Tumor evaluation (CT scan chest/abdomen/pelvis (or MRI) and bone scan)

10.2.5 Every subsequent 12 weeks:

- ECOG performance status
- A complete physical exam including vitals

- Laboratory assessments (hematology, chemistry, and PSA)
- Tumor evaluation to be performed every 12 weeks or whenever disease progression is suspected based on the investigator's clinical assessment of patient symptoms and laboratory data (CT scan chest/abdomen/pelvis (or MRI) and bone scan)

10.2.6 At Progression (See 5.3 for definition):

- ECOG performance status
- A complete physical exam including vitals
- Laboratory assessments (hematology, chemistry, PT/INR, APTT, PSA and Investigational samples)
- Tumor evaluation (CT scan chest/abdomen/pelvis (or MRI) and bone scan) within 30 days prior to biopsy.
- All subjects will undergo a biopsy of a metastatic lesion as described in Section 5.2 and Appendix B.
- Patients should have a PSA drawn on the day of taking their last dose of enzalutamide (e.g. day of biopsy for those undergoing a progression biopsy procedure or day of progression visit when enzalutamide is discontinued.)

10.3 Follow-up

After discontinuing enzalutamide, a follow-up PSA should be drawn prior to initiating the next therapy (ideally, 2-3 weeks after discontinuing enzalutamide treatment) in those patients who are agreeable. In those who have not initiated a new treatment of their prostate cancer (radiation, local surgery, or systemic therapy) by 6 weeks, a PSA should be drawn then in patients who are agreeable.

After termination of study participation, subjects will be followed using these guidelines:
10.3.1. If a patient discontinues Enzalutamide treatment for any reason other than Radiographic Progression or Clinical Progression as defined in section 5.2, the patient will continue to be followed until criteria for Radiographic or Clinical Progression is met. Tumor evaluations should follow the same schedule as on study (every 12 weeks). The patient will also be followed for stabilization or resolution of any study-related toxicities.

After progression, all patients will be followed by telephone, doctor visit, or medical record review every 12 weeks for survival status.

10.4 Schedule of Events

	Screening	Enrollment	Day 1 (Start Enzalutamide)	Week 6	Week 12	Every 12 Weeks	At Progression or Time Recommended for Drug to be Discontinued (See 5.3)	Follow up lab work (see 10.3)	Follow up: Every 12 Weeks
	Within 35 days of Enrollment	Within 10 days of Day 1		± 3 Days	± 8 Days	± 8 Days	± 8 Days		± 8 Days
Enzalutamide (provided)			X		X	X			
Biopsy		X					X[d]		
Informed consent	X								
Medical history	X								
Concurrent medications	X		X	X	X	X			
Physical exam	X		X	X	X	X	X		
Vital signs	X		X	X	X	X	X		
Performance status	X		X	X	X	X	X		
Hematology[f]	X[i]		X	X	X	X	X		
Chemistry[f]	X		X	X	X	X	X		
PT/INR & APTT	X[i]						X[d]		
PSA	X		X	X	X	X	X[e]	X[h]	
Testosterone	X								
LDH[g]	X								
Investigational samples for banking[a]							X		
Adverse event evaluation		X	X	X	X	X	X		
Radiologic evaluation	X[c]				X	X[b]	X[d]		
Telephone contact for Vital Status									X

a: See Appendix C for collection and processing instructions
b Radiologic evaluations will be done every 12 weeks or when progression is suspected
c: Scans must be performed within 30 days of the baseline biopsy
d: Progression biopsy, Progression PT/INR & APTT, and Radiologic Evaluations must be done within 30 days of Progression or time recommended for drug to be discontinued
e. Patients should have a PSA drawn on the day of taking their last dose of enzalutamide (e.g. day of biopsy for those undergoing a progression biopsy procedure or day of progression visit when enzalutamide is discontinued.
f. Chemistry: Na, K, Cl, CO2, BUN, Creat, Ca, Glu, Alb, Alk Phos, T. Bili, AST, T. Protein, ALT
Hematology : WBC count with differential, platelet count, hemoglobin, and hematocrit
g: An LDH may be drawn at any point prior to study drug administration (i.e screening period and on day 1)
h. After discontinuing enzalutamide, a follow-up PSA should be drawn prior to initiating the next therapy (ideally, 2-3 weeks after discontinuing enzalutamide treatment) in those patients who are agreeable. In those who have not initiated a new treatment of their prostate cancer (radiation, local surgery, or systemic therapy) by 6 weeks, a PSA should be drawn then in patients who are agreeable.
i. Hematology and PT/INR and APTT should be performed no more than 14 days prior to biopsy

11.0 MEASUREMENT OF EFFECT

11.1 PSA Response PSA Changes

11.1.1 PSA Response: A ≥ 50% reduction at 12 weeks after the initiation of therapy vs. baseline. Baseline PSA will be defined as the measurement obtained immediately prior to initiation of Enzalutamide on Day 1 of study.

11.1.2 Waterfall plots will be used to report the percentage change in PSA from baseline vs. 12 weeks after starting Enzalutamide treatment for each patient. Waterfall plots will also be used to report the maximal PSA decline at any point on study for each patient. Baseline PSA will be defined as the measurement obtained immediately prior to initiation of Enzalutamide on Day 1 of study.

11.1.3 PSA Progression (per PSAWG2 guidelines)[35]:

11.1.3.1 If PSA has declined from baseline, progression is defined as time from start of therapy to first PSA increase that is ≥ 25% and ≥ 2 ng/mL above the nadir, and which is confirmed by a second value 3 or more weeks later

11.1.3.2 If there is no decline from baseline, progression is defined as PSA increase ≥ 25% and ≥2 ng/mL after 12 weeks, which is confirmed by a second value 3 or more weeks later.

Nadir will be defined as the lowest PSA value that was confirmed by a second equal or lower measurement. (Thus the nadir PSA is the second lowest PSA value measured.)

11.1.4 Stable PSA: Does not meet criteria for response or progression.

11.2 Antitumor Effect – Solid Tumors

For the purposes of this study, subjects should be re-evaluated for response every 12 weeks.

Objective radiographic response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

11.2.1 Definitions

Evaluable for toxicity: Any subject, who undergoes a biopsy, whether or not he receives subsequent study drug treatment, will be analyzed for toxicity related to the biopsy.

Evaluable for objective response: Only those subjects who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These subjects will have their response classified according to the definitions stated below. (Note: Subjects who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response: Subjects who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease response. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

11.2.2 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded for non-nodal lesions and short axis for nodal lesions) as ≥20 mm by chest x-ray, as ≥10 mm with CT scan, or ≥10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).
Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable.

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

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

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

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

11.2.3 Methods for Evaluation of Measurable Disease

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

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam. Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

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

11.2.4 Radiographic Response Criteria

11.2.4.1 Evaluation of Target Lesions

Complete Response (CR):	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
Partial Response (PR):	At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.
Progressive Disease (PD):	At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).
Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

11.2.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).
Note: If tumor markers are initially above the upper normal limit, they must normalize for a subject to be considered in complete clinical response.

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

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

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

11.2.4.3 Evaluation of Best Overall Radiographic 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 subject's best response assignment will depend on the achievement of both measurement and confirmation criteria.

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

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

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

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

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

11.2.5 Bone Scan Interpretation

Bone scan progression is defined as the appearance of ≥ 2 unequivocally new lesions. For the first scheduled assessment only, a confirmatory scan performed 6 or more weeks later must show a minimum of 2 or more additional new lesions. If changes on bone scan are thought to be due to flare or due to another cause, i.e, injury, they will not be considered progression. Bone scans should be interpreted in the context of the entire clinical status of the patient. The date of progression is the date of the first scan that shows the change.

11.2.6 Progression-Free Survival

Progression-free survival is defined as time from Day 1 of study drug treatment to date of first documented radiographic or clinical progression (see Sec 5.3)..

11.2.7 Overall Survival

Time to death of all causes will be calculated from Day 1 to the date of death from any cause.

11.2.8 Disease-Specific Survival

Time to death from prostate cancer will be calculated from Day 1 to the date of death from prostate cancer if this is the cause of death.

12.0 DATA REPORTING/REGULATORY REQUIREMENTS

12.1 Protocol Review

The protocol and informed consent form for this study at OHSU must be reviewed and approved in writing by the OHSU Knight Cancer Institute (CI) Clinical Research Review Committee (CRRC) and the appropriate Institutional Review Board (IRB) prior to any subject being consented on this study.

All sites must have IRB approval of protocol by the IRB of record before consenting any subjects.

12.2 Informed Consent

Written informed consent will be obtained from all subjects, or the legally authorized representative of the subject, participating in this trial, as stated in the Informed Consent section of the case of Federal Regulations, Title 21, Part 50. If a subject's signature cannot be obtained, and for all subjects under the age of 18, the investigator must ensure that the

informed consent is signed by the subject's legally authorized representative. Documentation of the consent process and a copy of the signed consent shall be maintained in the subject's medical record.

All sites must have IRB approval of ICF by the IRB of records before consenting any subjects.

12.3 Changes to Protocol

Any modification of this protocol must be documented in the form of a protocol revision or amendment signed by the principal investigator and approved by the CRRC and IRB, before the revision or amendment may be implemented. The only circumstance in which the amendment may be initiated without regulatory approval is for a change necessary to eliminate an apparent and immediate hazard to the subject. In that event, the investigator must notify the CRRC and IRB in writing within 10 working days after the implementation. Investigators holding the IND must notify FDA of substantive changes to the protocol.

A participating site must submit proposed changes to protocol to the OHSU Coordinating Center for review and endorsement before participating site may implement changes.

12.4 Maintenance of Records

If the investigator relocates or for any reason withdraws from the study, the study records must be transferred to an agreed upon designee, such as another institution, another investigator, or to OHSU Knight Cancer Institute Clinical Trials Office. Records must be maintained according to sponsor or FDA requirements.

12.5 OHSU IRB Reporting of Unanticipated Problems and Adverse Events

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.6 OHSU Knight Cancer Institute Data and Safety Monitoring Plan

In addition to complete study and pharmacy files, complete records must be maintained on each subject treated on this protocol. OHSU Knight Cancer Institute, through the auditing function of the Knight Clinical Trials Office, is responsible for ensuring that all member investigators and affiliate investigators conduct clinical research studies in compliance with local IRB standards, FDA regulations and NIH policies and in accordance with the Data and Safety Monitoring Plan policies and procedures
http://ozone.ohsu.edu/cancer/sharedres/kctoresdocs.cfm
Locally initiated studies will be audited by OHSU Knight CI Auditor. Newly approved studies may be audited any time after enrollment has been initiated. Each OHSU Knight approved treatment protocol will be audited on an annual basis in accordance with the Knight Data and Safety Monitoring Plan.

It is the responsibility of each participating site's principal investigator to ensure that the study isconducted in compliance with local IRB standards, FDA regulations, and NIH policies. It is also the responsibility of each site's principal investigator to ensure that quality assurance audits at their site are conducted according to their institution's policies and procedures. The quality assurance audit process provides assurance that reported data accurately reflects the data in the primary subject record.

12.7 Inclusion of Women, Minorities and Children

12.8.1 Inclusion of Women and Minorities

No OHSU Knight Cancer Institute study will focus on any particular racial or ethnic subset. No subject will be excluded from the study on the basis of racial or ethnic origin. Male and minority volunteers will be recruited for this study from the general population and 100% men will be studied.

The racial and ethnic composition of the study should represent that of the state of Oregon (see Table 1). If the prevalence of the disease being studied is consistent across race, ethnicity and gender, then these figures can be used to calculate projected enrollments in Table 2. If the disease being studied does *not* affect both genders or all races and ethnicities equally (e.g., cervical cancer only affects women and Black males are more likely than White males to have prostate cancer), then this information should be taken into account when calculating the projected enrollment. The OHSU General Clinical Research Center has links to various sources of statistics on their webpage at www.ohsu.edu/gcrc. If a different source is used in calculating projected enrollments, that source should be cited below Table 2.

Table 1: Population Demographics - Oregon (%)

Ethnic Category	**Sex/Gender**		
	Females	Males	Total
Hispanic or Latino			11.7
Not Hispanic or Latino			88.3
Ethnic Category: Total of all subjects*			100*
Racial Category			
American Indian or Alaskan Native			1.4
Asian			3.7
Black or African American			1.8
Native Hawaiian or other Pacific Islander			0.3
White			83.6
More than one race			3.8
Unknown/Other			5.3
Racial Category: Total of all subjects*			100*
TOTALS	50.4	49.6	100*

Source: U.S. Census Bureau, 2010 *Totals may not equal 100 due to rounding.

Table 2: Projected Accrual for the Present Study

Ethnic Category	Sex/Gender			
	Females	Males	Unknown	Total
Hispanic or Latino	0	4	0	
Not Hispanic or Latino	0	32	0	
Unknown	0	0	0	
Ethnic Category: Total of all subjects*	0	36	0	36*
Racial Category				
American Indian or Alaskan Native	0	0-1	0	
Asian	0	0-2	0	
Black or African American	0	0-1	0	
Native Hawaiian or other Pacific Islander	0	0-1	0	
White	0	30	0	
More than one race	0	0-2	0	
Unknown	0	0-2	0	
Racial Category: Total of all subjects*	0	36	0	36*

Source: Adapted from U.S. Census Bureau, 2010 *Totals may not equal 100 due to rounding.

12.8.2 Inclusion of Children

This protocol does not include children for the following reason: Prostate cancer does not affect children under the age of 18.

13.0 STATISTICAL CONSIDERATIONS

13.1 Study Design

This is a prospective, single-arm multi-center study to identify molecular markers that are predictive of the degree and duration of disease control or overall survival with Enzalutamide treatment in men with metastatic CRPC. We expect to enroll 36 patients over the period of three years, with an approximate of 12 patients per year. All subjects will be treated with Enzalutamide and followed on protocol until disease progression or intolerance. Two tumor biopsies may be conducted: the first biopsy at baseline before Enzalutamide treatment for all enrolled patients, followed by the second biopsy at progression.

13.2 Primary and Secondary Objectives and Endpoints

Primary Objective and Primary Endpoint
To assess the correlations between baseline molecular features and pathways and PSA change (</≥ 50% decline) at 12 weeks vs. baseline.

Secondary Objectives
The secondary objectives are:

To measure PSA change at 12 Weeks and at each study visit vs. baseline after enzalutamide

treatment.

To measure objective response defined in Section 11.1.1 after enzalutamide treatment

To assess the correlations between the baseline molecular features and pathways and Progression-Free Survival (defined as time from Day 1 of study drug treatment to date of first documented radiographic progression or clinical progression- See Sec 5.3), Disease-Specific Survival (defined as the time from Day 1 of study drug to date of death from prostate cancer), and Overall Survival (defined as time from Day 1 of study drug treatment to date of death from any cause).

To assess the correlations between the baseline molecular features and pathways and time to PSA progression (see Sec 11.1).

To identify molecular features and cellular pathways present in tumors from men with metasatic CRPC that are progressing despite Enzalutamide treatment.

To explore correlation between baseline molecular features and pathways and changes in Circulating Tumor Cells (CTCs) counts defined in Sec 11.3.1.

To explore correlation between baseline molecular features and pathways and objective response defined in Section 11.1.1.

To assess the correlations between the baseline molecular features and pathways and degree of PSA decline at 12 weeks and maximal PSA decline observed while on study.

To assess the correlations between the baseline molecular features and time on treatment.

13.3 Analysis Populations

For the primary endpoint, evaluable patients will include those patients for whom data from genomic studies and IHC tests are available from the pre-treatment biopsy samples and who have completed at least 12 weeks of therapy or who have confirmed disease progression prior to 12 weeks of therapy. For the secondary endpoint of time to PSA progression, evaluable patients will include those for whom PSA data are available at follow-up timepoints on study. For the secondary endpoint of mechanisms at disease progression/drug discontinuation, evaluable patients will include those patients for whom data from genomic studies and IHC tests are available from both the pre-treatment and post-treatment biopsy samples. Any patient who undergoes a biopsy, whether or not he receives subsequent study drug treatment, will be analyzed for safety.

13.4 Statistical Analysis Plan

Analysis for Primary Objective

The proportion of patients with or without a $\geq$50% decline in PSA values at 12 weeks will be reported with 95% exact confidence interval

Simple Logistic regression model will be used to evaluate the association between PSA responseand each of the potential molecular biomarker predictors, including (gene expression signatures, copy number alterations, mutations, IPLs (integrated pathway levels and IHC measurements). All continuous variables with skewed distributions will be log transformed and centered before incorporation. Based on the logistic regression model, a receiver-operating characteristic (ROC) curve will be generated and the area under the curve (AUC) will be calculated with its 95% confidence interval. The ROC curve will also be used to help determine an optimal cutoff for each molecular predictor for a most accurate prediction of PSA response..
Depending on the actual number of patients who are Enzalutamide-responsive or resistant, a multivariable logistic model may be developed, starting with all covariates that have a p-value < 0.25 from the Simple Logistic regression model. The model selection will be based on the Akaiki Information Criteria (AIC), and the c-index will be reported with the final model. If the number of patients who are Enzalutamide responsive or resistant is not big enough (less than 10 for each 1 degree of freedom in the model) for the multivariate modeling, we will leave the multivariate modeling procedure for future studies.

Analysis for Secondary Objectives

We will use Random Forests classification to identify molecular features and pathways present in patients with disease progression or who discontinue Enzalutamide treatment. Analyses will be conducted in R using the randomForest library. Forests will be created with at least 10,001 trees (odd number ensures fully deterministic model) and otherwise default settings. We will perform 50 replicates with five-fold CV and step of 0.9 on a log scale. The number of predictors with minimum mean CV error across the replicates will be chosen as the optimal predictor number. We will assess performance by ROC AUC reported values for Random Forests internal out-of-bag (OOB) testing. The OOB testing is based on the fact that each tree in the forest is built on a random 2/3 subset of patients and the remaining 1/3 used as test set for that tree.

The association between molecular predictors and survival outcomes (e.g., PFS, DSS and OS) and time on treatment will be assessed using cox regression model. In addition, Kaplan-Meier plots will be used to graphically illustrate the survival distributions for PFS, DSS and OS across the strata of categorical molecular predictors. Logistic regression model will be used to assess the association between molecular predictors and response rate. Linear regression model will be used to assess the association for changes in CTC counts from baseline and maximal PSA observed while on study.

13.5 Sample Size and Power

Our main goal is to identify molecular features and pathways that would allow us predict which tumors will have durable disease control with Enzalutamide treatment.. Traditional power calculations cannot be used to determine the number of samples (ie, patients) required because the assumptions of independence and normality do not hold.[37] An alternative approach is to model this problem as a learning curve in which the classification error is characterized as an inverse power-law[38]: error = $an^{-\alpha} + b$, where error is the expected error rate given n training samples, a is the learning rate, α is the decay rate and b is the Bayes error, or minimum error achievable. This approach has been applied to several cancer classification problems, and estimates for the variables can be found in the literature.[38] For our purposes, we have used the average values from 7 cancer datasets to estimate a, α and b. If we let a = 0.736, α = 0.65 and b = 0.001, we can estimate the error for various sample sizes. We estimate that we can achieve an error rate of ~0.08 with 30

samples. Our prior work with metastatic biopsy collection in collaboration with Dr. Philip Febbo demonstrated that approximately 80% of patients' biopsies had sufficient material to perform gene expression profiling. Therefore, 36 patients should be sufficient to obtain approximately 30 patients with informative samples.

The Knight Cancer Institute Biostatistics Shared Resource will support these analytic methods both at the study design stage and during the data analysis stage of the project.

REFERENCES

1. Tannock IF, de Wit R, Berry WR, et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. N Engl J Med 2004;351:1502-12.
2. Chen CD, Welsbie DS, Tran C, et al. Molecular determinants of resistance to antiandrogen therapy. Nat Med 2004;10:33-9.
3. de Bono JS, Oudard S, Ozguroglu M, et al. Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial. Lancet 2010;376:1147-54.
4. Scher HI, Beer TM, Higano C, et al. Antitumour Activity of MDV3100 in a Phase 1-2 Study of Castration-Resistant Prostate Cancer. Lancet 2010;375:1437-46.
5. Tran C, Ouk S, Clegg NJ, et al. Development of a second-generation antiandrogen for treatment of advanced prostate cancer. Science 2009;324:787-90.
6. De Bono J. FK, Saad F., Taplin M., Sternberg C.N., Miller K, Mulders P, Chi K.N., Armstrong A.J., Hirmand M., Selby B, Scher H. Primary, secondary, and quality-of-life endpoint results from the phase III AFFIRM study of MDV3100, an androgen receptor signaling inhibitor. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2012;30.
7. Scher HI, Sawyers CL. Biology of progressive, castration-resistant prostate cancer: directed therapies targeting the androgen-receptor signaling axis. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2005;23:8253-61.
8. Taplin ME. Drug insight: role of the androgen receptor in the development and progression of prostate cancer. Nature clinical practice Oncology 2007;4:236-44.
9. de Bono JS, Logothetis CJ, Molina A, et al. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med 2011;364:1995-2005.
10. Guo Z, Yang X, Sun F, et al. A novel androgen receptor splice variant is up-regulated during prostate cancer progression and promotes androgen depletion-resistant growth. Cancer Res 2009;69:2305-13.
11. Hu R, Dunn TA, Wei S, et al. Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. Cancer Res 2009;69:16-22.
12. Sun S, Sprenger CC, Vessella RL, et al. Castration resistance in human prostate cancer is conferred by a frequently occurring androgen receptor splice variant. The Journal of clinical investigation;120:2715-30.
13. Carver BS, Chapinski C, Wongvipat J, et al. Reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN-deficient prostate cancer. Cancer Cell;19:575-86.
14. Mulholland DJ, Tran LM, Li Y, et al. Cell autonomous role of PTEN in regulating castration-resistant prostate cancer growth. Cancer Cell;19:792-804.
15. Gottlieb B, Beitel LK, Nadarajah A, Paliouras M, Trifiro M. The androgen receptor gene mutations database: 2012 update. Human mutation 2012;33:887-94.
16. Linja MJ, Savinainen KJ, Saramaki OR, Tammela TL, Vessella RL, Visakorpi T. Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. Cancer Res 2001;61:3550-5.
17. Visakorpi T, Hyytinen E, Koivisto P, et al. In vivo amplification of the androgen receptor gene and progression of human prostate cancer. Nat Genet 1995;9:401-6.
18. Ueda T, Bruchovsky N, Sadar MD. Activation of the androgen receptor N-terminal domain by interleukin-6 via MAPK and STAT3 signal transduction pathways. J Biol Chem 2002;277:7076-85.
19. Chang KH, Li R, Papari-Zareei M, et al. Dihydrotestosterone synthesis bypasses testosterone to drive castration-resistant prostate cancer. Proc Natl Acad Sci U S A 2011;108:13728-33.
20. Febbo PG, Thorner A, Rubin MA, et al. Application of oligonucleotide microarrays to assess the biological effects of neoadjuvant imatinib mesylate treatment for localized prostate cancer. Clin Cancer Res 2006;12:152-8.
21. Bild AH, Yao G, Chang JT, et al. Oncogenic pathway signatures in human cancers as a guide to targeted therapies. Nature 2006;439:353-7.

22. Pittman J, Huang E, Nevins J, Wang Q, West M. Bayesian analysis of binary prediction tree models for retrospectively sampled outcomes. Biostatistics 2004;5:587-601.
23. Memarzadeh S, Cai H, Janzen DM, et al. Role of autonomous androgen receptor signaling in prostate cancer initiation is dichotomous and depends on the oncogenic signal. Proc Natl Acad Sci U S A 2011;108:7962-7.
24. Thomas C, Zoubeidi A, Kuruma H, et al. Transcription factor Stat5 knockdown enhances androgen receptor degradation and delays castration-resistant prostate cancer progression in vivo. Molecular cancer therapeutics 2011;10:347-59.
25. Wang S, Gao J, Lei Q, et al. Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer. Cancer Cell 2003;4:209-21.
26. Thomas GV, Horvath S, Smith BL, et al. Antibody-based profiling of the phosphoinositide 3-kinase pathway in clinical prostate cancer. Clin Cancer Res 2004;10:8351-6.
27. Lamoureux F, Thomas C, Yin MJ, et al. Clusterin inhibition using OGX-011 synergistically enhances Hsp90 inhibitor activity by suppressing the heat shock response in castrate-resistant prostate cancer. Cancer Res 2011;71:5838-49.
28. Tanaka H, Kono E, Tran CP, et al. Monoclonal antibody targeting of N-cadherin inhibits prostate cancer growth, metastasis and castration resistance. Nat Med 2010;16:1414-20.
29. Suwaki N, Vanhecke E, Atkins KM, et al. A HIF-regulated VHL-PTP1B-Src signaling axis identifies a therapeutic target in renal cell carcinoma. Sci Transl Med 2011;3:85ra47.
30. Tariq MA, Kim HJ, Jejelowo O, Pourmand N. Whole-transcriptome RNAseq analysis from minute amount of total RNA. Nucleic acids research 2011;39:e120.
31. Tewhey R, Warner JB, Nakano M, et al. Microdroplet-based PCR enrichment for large-scale targeted sequencing. Nature biotechnology 2009;27:1025-31.
32. Vaske CJ, Benz SC, Sanborn JZ, et al. Inference of patient-specific pathway activities from multi-dimensional cancer genomics data using PARADIGM. Bioinformatics 2010;26:i237-45.
33. Efron B, Tibshirani, R. On testing the significance of sets of genes. Ann Appl Stat 2007;1.
34. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci U S A 1998;95:14863-8.
35. Scher HI, Halabi S, Tannock I, et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2008;26:1148-59.
36. Danila DC, Fleisher M, Scher HI. Circulating tumor cells as biomarkers in prostate cancer. Clin Cancer Res 2011;17:3903-12.
37. Pusztai L, Hess KR. Clinical trial design for microarray predictive marker discovery and assessment. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO 2004;15:1731-7.
38. Mukherjee S, Tamayo P, Rogers S, et al. Estimating dataset size requirements for classifying DNA microarray data. Journal of computational biology : a journal of computational molecular cell biology 2003;10:119-42.

APPENDIX A

ECOG PERFORMANCE STATUS*	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

* As published in Am. J. Clin. Oncol.:

Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

APPENDIX B

BIOPSY PROCEDURE

The tumor biopsies for this study will be collected and shipped via the University of California, San Francisco, protocol CC#125519 (OHSU protocol #9204) -Radiologically Guided Biopsies Of Metastatic Castration Resistant Prostate Cancer To Identify Adaptive Mechanisms of Resistance.

Critical to the application of the AR signature, is the collection of prostate cancer tissue for genomic analysis. Due to the nature of metastatic prostate cancer, most biopsies will be from bone. Bone biopsies present unique challenges with respect to the collection and processing. However, regardless of collection technique, rapid freezing of the biopsy material is critical to subsequent success. As part of the trial, we will provide kits to each site enrolling patients so as to facilitate a standardized collection of tissue.

Patients are positioned by radiology and conscious sedation may be administered to improve patient comfort. This is based on the judgment of the interventional radiologist and may be restricted in some centers due to the availability of supportive services.

During the image-guided biopsy procedure we recommend the following general rules when choosing a metastatic site and a specific location within the metastatic tumor for biopsy:

1. If the patient has bone only disease, the pelvis is the preferred site for biopsy.
2. Any biopsy kit capable of obtaining core biopsies from bone is acceptable. The Bonopty Coaxial biopsy system with eccentric drill (http://www.vasocare.co.kr/product04-5.html).http://www.vasocare.co.kr/product04-5.html) is a recommended system. Internal diameter is 1.3mm. This is the smallest for collection of usable material.
3. In the bone, extremely blastic lesions seldom yield usable material. Yields are greater if biopsies are performed in marrow of abnormal signal intensity directly adjacent to blastic lesions. Often, we pass the needle tangential to the blastic lesion in the marrow space for our most successful collections.
4. In soft tissue, do not biopsy from regions of metastatic lesions that appear necrotic on CT or MRI due to extremely poor yield.
5. It is important that excessive compressive force is not required or used to expel the biopsy from the biopsy needle. This obscures cellular morphology. If it is difficult to expel the biopsy from the core needle, alternative biopsy kits should be used.

Please follow the directions below when processing the biopsy material:

Required Materials

Included in Kit:

- Disposable cryo-molds 15x15x5mm
- Sakura Tissue-Tek OCT compound
- Metal plate or tray
- Sarstedt black permanent marker
- Tissue-Tek Mega-Cassettes
- Styrofoam cooler (part of shipment box, a new box will be returned once the biopsies arrive at UCSF)
- Container of neutral buffered formalin (2 provided, keep extra, an additional vial will be included with the new box)

*Keep all remaining materials after procedure is completed for use on future biopsies unless otherwise noted

Not Included (should be available on site)

- Dry Ice (pellet form, smaller pellet size works best)
- Syringe needle (core manipulation; ≤19g)
- Forceps (fine toothed)

Protocol for Biopsies

1. Prepare a cold working surface by filling the supplied styrofoam cooler container roughly half full of dry ice. Shake the container to form a fairly even layer.

2. Place the metal sheet or tray into the cooler and allow it to cool below -10°C. Temperatures below this point will freeze OCT compound quickly, facilitating the block formation. Having a flat metallic surface is also important.

3. Prior to heading to the collection, place several cryomolds on the cold metal surface allowing them to cool down prior to the biopsy.

4. Label 5 tissue cassettes, then place on dry ice to pre-chill before use during the procedure.

5. Take the cooler setup to the location of the biopsy and be sure to have easy access to it during the procedure. Be sure to take the formalin vial in the zip-lock bag as well.

6. Once the radiologist says a core is about ready, fill one of the cryomold trays roughly half-full of OCT so that the compound is partially frozen by the time the core is added. The OCT compound will appear opaque as it solidifies.

7. Transfer a core to the partially solidified OCT layer in the cryomold. The best method of tissue transfer from the biopsy needle to the pallet will depend on the type of needle used. The core biopsy can be directly placed into the cryomold of OCT, but care must be taken to ensure the core is lying as close to flat as possible as it freezes. Bonopty and other hollow core needles will yield a cylindrical core that is ejected via a plunger needle. The pressure created by the plunger directly onto the core can unpredictably eject the core from the needle. If the core cannot be placed directly on the OCT, it can be ejected onto a gauze pad and transferred immediately onto the OCT so as to minimize time for RNA degradation and protein de-phosphorylation. This helps to maintain needle sterility and allows for more careful manipulation of the core away from the sterile procedure cart. Manipulation of the core from gauze pad to cryomold is done with a syringe needle.

8. Positioning of the core is very important. Be sure to place the core flat down on the center of the OCT layer. The tissue can freeze on contact if the OCT layer has solidified, thus preventing manipulation once the tissue is on the OCT surface. Adding the core to OCT prior to solidification is ideal since the tissue and OCT will then freeze at the same time, resulting in better sectioning later. Layers frozen in different stages tend to cause separation during sectioning.
9. Immediately cover the core with OCT, working in a slow circular motion around the core such that OCT fills in the sides, then surrounds the top. Contact of the liquid OCT with the tissue core is important to preserve the tissue and facilitate in cryo sectioning later.

10. nce the OCT becomes opaque in each cryomold, the tissue block is ready to be transferred to the labeled cold cassette.

11. In addition to collecting fresh-frozen biopsy cores, 2 core must be collected for paraffin embedding. This core should be placed in the container of neutral buffered formalin. Close the jar tightly, and return to the zip-lock bag.

12. Package the biopsies into the provided insulated box. The cold cryo cassettes should be placed in dry ice in the insulated section of the box, and the formalin vial contained within the zip-lock bag should be placed outside of the insulated region of the box. The appropriate spot for the formalin vial will be labeled.

13. Include with the shipment, the sample shipment log (Appendix K) that should include comments regarding core quality (long cylindrical core, bone shards, mostly blood clot, etc), the quantity of cores and any irregularities in the freezing process. The technician performing the collection should be sure to note their unique patient ID, the biopsy date, and the time of the procedure as well.

14. Prior to any shipments, the study coordinator and designated laboratory staff at UCSF and OHSU must be notified. Notification should come in the form of email at least 2 – 3 days or, if possible, a week prior to an anticipated shipment. The email should consist of the date of scheduled biopsy, unique patient ID, participating site name and site contact; this should be sent to phillip.febbo@ucsf.edu and adam.foye@ucsf.edu and thomasge@ohsu.edu.

15. Mail by Express Overnight delivery to the address specified on the provided shipping labels. Unless special arrangements are made, do not ship on Fridays, Saturdays, or Sundays.

APPENDIX C

Optional, Investigational Sample Collection, Processing, and Storage

Investigational serum samples will be drawn using venipuncture at each time point dictated by the schedule of events (see 10.4). All supplies listed in this appendix, with the exception of the 2ml cryovials supplied by OHSU, are to be obtained locally by the sub-site.

Draw 10ml whole blood into an EDTA (lavender top) vacutainer tube. Process blood as soon as possible. It is recommended that the resulting plasma is frozen within one (1) hour of blood draw, and that no more than two (2) hours elapse from collection to freezing.

Centrifuge whole blood at 1,000g for 10 minutes at room temp. Using a polyethylene transfer pipet, transfer at least 1ml of the resulting plasma into each of the four (4) provided Cryovials. Be sure that the cells from the buffy coat are not removed with the plasma phase. Cap and label each Cryovial with patient study ID, type of sample (plasma), date of collection, and time point (e.g. day 1, week 12, off-study, etc.).

Freeze and store the samples in an upright position at -80°C until shipping to OHSU on dry ice. Samples may be stored on site and batch shipped to OHSU. Please alert the OHSU study coordinator listed below of any planed shipments to ensure proper receipt.

Follow these instructions to ensure proper transport/receipt of samples:

- Samples may only be shipped Monday through Thursday via overnight delivery. Do not ship samples on Fridays as OHSU is unable to receive packages on Saturdays.
- Place the frozen samples and some absorbent material in a plastic biohazard bag and seal the bag.
- Samples must be shipped in a Styrofoam insulated shipping container.
- Place enough **dry ice** to cover the bottom of the Styrofoam container, place bagged samples on top of dry ice, then cover with 7 to 10 pounds of dry ice.
- To ensure timely delivery, the package **MUST** be properly labeled to indicate the presence of dry ice and biological specimens. Consult a local shipping authority if unsure how to properly label.

INVESTIGATIONAL SAMPLES SHOULD BE SENT TO:

SPECIMEN MANAGER
OREGON HEALTH & SCIENCE UNIVERSITY
3303 SW Bond Avenue, mailcode: CH14R
PORTLAND, OR 97239
CONTACT INFORMATION:
PHONE: (503) 494-4396
FAX: (503) 494-6197

OHSU ONLY

In addition to the 10ml blood collected by all sites, the OHSU site only will collect an additional 30ml of blood in EDTA (lavender top) vacutainer tubes. Centrifuge blood at 1000g for 10 minutes at room temp. Transfer the plasma layer (~5ml per tube) to 1.5ml Eppendorf tubes in 1ml aliquots and centrifuge at 15,000g for 10 minutes at room temp. Transfer the 1ml plasma supernatants to labelled Cryovials. Dislodge the remaining buffy coat pellets from the Eppendorf tubes and combined them in one labeled Cryovial. Store all samples at -80C.

APPENDIX D

SAE REPORT COVER SHEET

IIT Investigator's Name:	
	(First, Last)
IIT Study Site Information	
Institution Name:	
City, State:	
IIT Study Number:	IIT000800
IIT Protocol Title:	
# of pages (including cover page)	

Please return this cover page along with the MEDWATCH form.

APPENDIX E

Below is a partial list of potent CYP inhibitors and inducers.

Potent CYP Inhibitors and Inducers

CYP Inhibitors	CYP Inducers
amiodarone	carbamazepine
atazanavir	Rifampin
clarithromycin	
disulfirum	
fluconazole	
fluoxetine	
fluvoxamine	
fluvoxamine	
gemfibrozil	
indinavir	
itraconazole	
ketoconazole	
moclobemide	
nefazodone	
nelfinavir	
omeprazole	
paroxetine	
quinidine	
ritonavir	
saquinavir	
telithromycin	

APPENDIX F

Drug Diary

Study: Molecular Mechanisms Underlying Tumor Progression Despite Enzalutamide Treatment **IRB#**: XXXX
Once the day 1 date is entered, the remaining dates will auto-populate in this form.

Last, First Name:			**MRN:**		**Study Period: Week X-X**	
Sunday	**Monday**	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Saturday**
			Enter date	**Enter date**	**Enter date**	**Enter date**
			Week Enzalutamide: ___ 160mg	Enzalutamide: ___ 160mg	Enzalutamide: ___ 160mg	Enzalutamide: ___ 160mg
Enter date	**Enter date**	**Enter date**	**Enter date**	**Enter date**	**Enter date**	**Enter date**
Enzalutamide: ___ 160mg	Enzalutamide: ___ 160mg	Enzalutamide: ___ 160mg	Enzalutamide: ___ 160mg	Enzalutamide: ___ 160mg	Enzalutamide: ___ 160mg	Enzalutamide: ___ 160mg
Enter date	**Enter date**	**Enter date**	**Enter date**	**Enter date**	**Enter date**	**Enter date**
Enzalutamide: ___ 160mg	Enzalutamide: ___ 160mg	Enzalutamide: ___ 160mg	Enzalutamide: ___ 160mg	Enzalutamide: ___ 160mg	Enzalutamide: ___ 160mg	Enzalutamide: ___ 160mg
Enter date	**Enter date**	**Enter date**	**Enter date**	**Enter date**	**Enter date**	**Enter date**
Enzalutamide: ___ 160mg	Enzalutamide: ___ 160mg	Enzalutamide: ___ 160mg	Enzalutamide: ___ 160mg	Enzalutamide: ___ 160mg	Enzalutamide: ___ 160mg	Enzalutamide: ___ 160mg
Enter date	**Enter date**	**Enter date**				
Enzalutamide: ___ 160mg	Enzalutamide: ___ 160mg	Enzalutamide: ___ 160mg				

* Drug Accountability: Please sign the calendar to indicate you have taken the above medication as instructed and return the signed calendar at the next study visit. Please note any missed doses and the reason missed on the specific date.

Patient Signature: ____ Date: ____

APPENDIX G

Partial list of medications which lower the seizure threshold. These medicine are not prohibited but should be used with caution.

abobotulinumtoxinA	HALOPERIDOL	PIMOZIDE
AMINOPHYLLINE	HYDROXYZINE	PROCAINE
AMITRIPTYLINE	IMIPENEM	PROCARBAZINE
AMPHETAMINE	IMIPRAMINE	PROCHLORPERAZINE
ATROPINE	IPRATROPIUM BROMIDE	PROCYCLIDINE
BACLOFEN	ISOCARBOXAZID	PROMETHAZINE
BENZTROPINE MESYLATE	ISONIAZID	PROPANTHELINE BROMIDE
BIPERIDEN	LIDOCAINE	PROPOFOL
BUPIVACAINE	LINDANE	PROPOXYPHENE
BUPROPION	LITHIUM	PROTRIPTYLINE
CHLORAMBUCIL	LOXAPINE SUCCINATE	RASAGILINE MESYLATE
CHLORPROMAZINE	MEFENAMIC ACID	RISPERIDONE
CITALOPRAM HYDROBROMIDE	MEPENZOLATE BROMIDE	SCOPOLAMINE
CLOMIPRAMINE	MEPERIDINE	SELEGILINE
CLOZAPINE	MEROPENEM	SERTRALINE
CYCLOSPORINE	METHSCOPOLAMINE BROMIDE	SOLIFENACIN SUCCINATE
DARIFENACIN HYDROBROMIDE	METHYLPHENIDATE	SUMATRIPTAN
DESIPRAMINE	METRONIDAZOLE	THEOPHYLLINE
DICYCLOMINE	NALIDIXIC ACID	THIETHYLPERAZINE
DORIPENEM	NEFAZODONE	THIORIDAZINE
DOXEPIN	NORTRIPTYLINE	TIOTROPIUM BROMIDE MONOHYDRATE
DULOXETINE	ORPHENADRINE CITRATE	TOLTERODINE
ERTAPENEM SODIUM	OXYBUTYNIN	TRAMADOL
ESCITALOPRAM OXALATE	OXYTOCIN	TRANYLCYPROMINE SULFATE
FENTANYL	PAROXETINE	TRIFLUOPERAZINE
FLUOXETINE	PENICILLIN V POTASSIUM	TRIHEXYPHENIDYL
FLUPHENAZINE	PENTAZOCINE LACTATE	TRIMIPRAMINE MALEATE
FLUVOXAMINE MALEATE	PERPHENAZINE	TROSPIUM CHLORIDE
GLYCOPYRROLATE	PHENELZINE SULFATE	

Appendix H

OHSU guidelines for drugs to avoid pre-biopsy

Drugs to avoid pre-biopsy	Length of washout pre-biopsy
COUMADIN	Withhold for at least 5 days prior to biopsy; consider bridge with heparin; restart ≥ 24 hours after biopsy
CLOPIDOGREL	Withhold for at least 7 days prior to biopsy
PRASUGREL	Withhold for at least 7 days prior to biopsy
TICARGRELOR	Withhold for at least 5 days prior to biopsy
FRACTIONATED HEPARIN	Withhold for at least 2-4 hours prior to biopsy
DABIGATRAN (PRADAXA)	Withhold for at least 2 days prior to biopsy if eGFR >50 or for at least 3 days prior to biopsy if eGFR <50; restart ≥ 24 hours after biopsy
RIVAROXABAN (XARELTO)	Withhold for at least 24 hours prior to biopsy; restart ≥ 24 hours after biopsy
APIXABAN (ELIQUIS)	Withhold for at least 24 hours before biopsy; restart ≥ 24 hours after biopsy
FONDAPARINUX (ARIXTRA)	Withhold for at least 3 days prior to biopsy
TICLOPIDINE (TICLID)	Withhold for at least 7 days prior to biopsy
ASPIRIN	Withhold for at least 5 days prior to biopsy
DOCLOFENAC (VOLTERAN)	Withhold for at least 24 hours prior to biopsy
IBUPROFEN	Withhold for at least 24 hours prior to biopsy
INDOMETHACIN	Withhold for at least 24 hours prior to biopsy
NAPROXEN	Withhold for at least 4 days prior to biopsy
PIROXICAM	Withhold for at least 11 days prior to biopsy
AGGRENOX	Withhold for at least 5 days prior to biopsy