TITLE: Phase II trial of enzalutamide for castrate-resistant prostate cancer (CRPC) with correlative assessment of androgen receptor (AR) signaling and whole-exome and transcriptome sequencing

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Sponsor-Investigator:

[redacted]

Co-Principal Investigators:

[redacted]

Coordinating Center:
Dana-Farber Cancer Institute

[redacted]

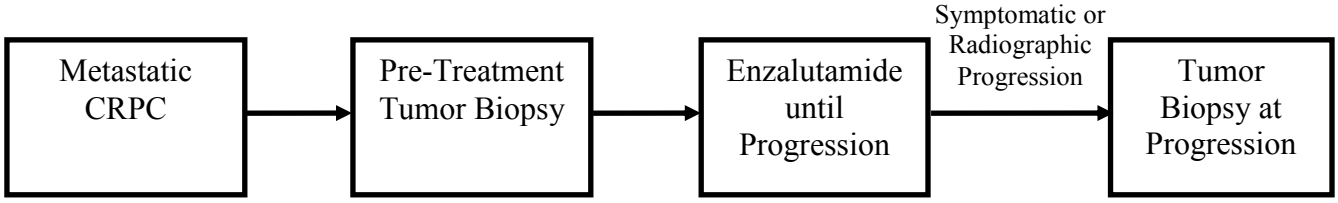
Agent(s):
Enzalutamide (IND # 74563)
IND/IDE held by: Medivation Inc.
Drugs, Biologics, Devices Provided by: Medivation Inc.

ABBREVIATIONS

Activation Function – AF
Adverse event – AE
Alanine transaminase – ALT
American Society of Anesthesiologists – ASA
ANC – Absolute neutrophil count
Androgen deprivation therapy – ADT
Androgen Receptor – AR
Androgen Response Element – ARE
Area under the concentration-time curve – AUC
Aspartate transaminase – AST
Blood Urea Nitrogen – BUN
Cancer Therapy Evaluation Program – CTEP
Case Report Form – CRF
Castration resistant prostate cancer – CRPC
Circulating Tumor Cells – CTCs
Code of Federal Regulations – CFR
Complete response – CR
Partial response – PR
Computed tomography – CT
Confidence interval – CI
Ligand binding domain – LBD
Dana-Farber Cancer Institute – DFCI
Dana-Farber/Harvard Cancer Center – DF/HCC
Dana-Farber/Partners Cancer Care – DF/PCC
Data Safety and Monitoring Committee – DSMC
Dehydroepiandrosterone – DHEA
Dehydroepiandrosterone-sulfate – DHEA-S
Deoxyribonucleic acid – DNA
Diastolic blood pressure – DBP
Dihydrotestosterone – DHT
DNA binding domain – DBD
Eastern Cooperative Oncology Group – ECOG
Echocardiogram – ECHO
Electrocardiogram – EKG
Fluoro-dihydrotestosterone-positron emission tomography – FHDT-PET
Food and Drug Administration – FDA
Good Clinical Practice – GCP
Half-life – $t_{1/2}$
Hazard ratio – HR
Health Insurance Portability and Accountability Act – HIPAA
Heat Shock Protein – HSP
Hematoxylin and eosin – H&E
Human immunodeficiency virus – HIV
Immunohistochemistry – IHC

Institutional Review Board – IRB
International Normalized Ratio – INR
Investigational New Drug – IND
Laser capture microdissection – LCM
Liver function test – LFT
Luteinizing hormone-releasing hormone – LHRH
Magnetic Resonance Imaging – MRI
Maximum plasma concentration – C_{max}
Messenger RNA – mRNA
Systolic blood pressure – SBP
Minimum plasma concentration – C_{min}
Multi Gated Acquisition Scan – MUGA
National Cancer Institute Common Terminology Criteria for Adverse Events – NCI CTCAE
New York Heart Association – NYHA
Not Reached – NR
N-Terminal Domain – NTD
Office of Data Quality- ODQ
Office for Human Research Studies – OHRS
Overall survival – OS
Partial thromboplastin time – PTT
Positron emission tomography – PET
Principal Investigator – PI
Progression free survival – PFS
Progressive disease – PD
Prostate specific antigen – PSA
Prostate Cancer Working Group – PCWG
Prothrombin time – PT
Response Evaluation Criteria In Solid Tumors – RECIST
Reverse transcription polymerase chain reaction – RT-PCR
Ribonucleic acid – RNA
Serious adverse event – SAE
Single-nucleotide polymorphism – SNP
Skeletal Related Event – SRE
Single-photon emission computed tomography - SPECT
Stable disease – SD
Time to PSA Progression – TTPP
Unknown – UN
Upper limit of normal – ULN
WBC – White blood cell count

SCHEMA



1. Population: Metastatic CRPC patients.

2. Treatment: Participants will be treated with enzalutamide in 28-day cycles. Participants will be continued on the study until symptomatic or radiographic progression.

3. Planned Enrollment: 66 participants across multiple sites.

4. Study Duration:
    a. Enrollment – one year.
    b. Sample collection and analysis – two years.
    c. Final report – 3-6 months after the end of data collection.

5. Data Safety Monitoring Committee (DSMP)/Safety Review: Quarterly.

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1. OBJECTIVES

The primary objective of this trial is to analyze possible AR related mechanisms of resistance to enzalutamide. Samples of CRPC will be obtained at baseline and at progression on treatment with enzalutamide. Tumor analysis will include AR sequencing (mutations, splice-variants), AR regulated gene expression, tumor androgen levels, profiling of enzymes involved in androgen synthesis/metabolism, and whole-exome and transcriptome sequencing. Secondary objectives include assessment of clinical response and correlation of clinical response to alterations in the AR signaling axis. Exploratory analyses will be to analyze circulating tumor cells (CTCs) for AR nuclear localization, AR splice variant expression and AR sequence. Additionally, we will analyze circulating tumor DNA.

1.1 Study Design

This is a phase II, prospective, multicenter, single-arm study accessing mechanisms of resistance to enzalutamide in participants with metastatic CRPC.

1.2 Primary Objectives

- To analyze possible AR related mechanisms of resistance to enzalutamide in serial CRPC biopsies including AR sequencing (mutations, splice variants), AR regulated gene expression, tumor androgen levels, profiling of enzymes involved in androgen synthesis/metabolism, and whole-exome and transcriptome sequencing.

1.3 Secondary Objectives

- Assess changes in serum androgen concentrations (including testosterone, dihydrotestosterone (DHT), and androgen precursors) between baseline and subsequent assessment visits.

- To assess prostate specific antigen (PSA) response to enzalutamide.

- To assess PSA response duration to enzalutamide.

- To assess response of measurable disease to enzalutamide.

- To assess duration of response of measurable disease to enzalutamide.

- To assess safety and tolerability of enzalutamide.

- To correlate alterations in AR mutation, AR splice variants, TMPRSS2/ERG, AR regulated gene expression, tumor androgens, serum androgens, whole-exome and transcriptome sequencing with PSA and radiographic response to enzalutamide.

- To investigate subsequent lines of therapy, including line of agent, name of agent, and PSA kinetics following study drug discontinuation and correlate with response to study drug.

1.4 Exploratory Objectives

- To measure circulating tumor cells (CTCs) as a marker of response to enzalutamide.
- To analyze CTCs for mechanisms of AR resistance including AR nuclear localization, AR splice variant expression and AR sequence.
- To analyze circulating tumor DNA for mechanisms of AR resistance and correlate to response to enzalutamide.
- To explore the association between quantitative ^{99m}Tc-MDP uptake in skeletal metastases, measured as activity concentration on quantitative SPECT/CT, with anatomical bone findings on CT, laboratory tumor markers (e.g. PSA, circulating tumor DNA) and overall response in an imaging substudy of patients enrolled at the Dana-Farber Cancer Institute only.

2. BACKGROUND

2.1 Study Agent(s)

2.1.1 Enzalutamide

Enzalutamide, formerly known as MDV3100, is a rationally-designed second generation AR inhibitor which functions by blocking several steps in the AR signaling cascade. Enzalutamide competitively binds the AR with great potency. Additionally, enzalutamide inhibits nuclear translocation of activated AR and inhibits the association of activated AR with DNA.[1]

Rational for Using Enzalutamide in Prostate Cancer
First generation antiandrogens, including bicalutamide, nilutamide, and flutamide, are reversible inhibitors of the AR and have a several-fold lower affinity to the AR compared with androgens. These agents have been used in the management of advanced prostate cancer for decades. Addition of an antiandrogen at the time of CRPC has been shown to lower PSA by 50% or more in approximately one-quarter of patients.[2-4] There is no data supporting the superiority of one antiandrogen over another, however nilutamide has been shown to induce PSA declines in men who have developed resistance to AR inhibition with flutamide or bicalutamide.[5]

It was first noted in the early 1990s that disease progression, despite the combination of an LHRH (luteinizing hormone-releasing hormone) agonist and an antiandrogen, could be stopped and reversed simply through discontinuation of the antiandrogen. Stopping the antiandrogen in men with a rising PSA, termed antiandrogen withdrawal, can result in PSA declines in between 10-20% of men.[6] Further work demonstrated that in the setting of AR overexpression or mutation, conventional antiandrogens have the potential to exhibit paradoxical partial agonist activity, promoting prostate cancer progression.[7] Thus, more potent antagonists lacking agonist activity are necessary.

Preclinical Data with Enzalutamide
Using the nonsteroidal agonist RU59603 as the parent scaffold compound, Sawyers and colleagues identified two oral diarylthiohydantoins, RD162 and enzalutamide, from a screen of nonsteroidal antiandrogens that retain antiandrogen activity in the setting of increased AR expression.[1] Both compounds have enhanced affinity for the AR (5-8 fold) compared to the antiandrogen bicalutamide. Enzalutamide competitively binds the AR with an IC_{50} of 36 nM compared to 160 nM for bicalutamide. Additionally, enzalutamide inhibits nuclear translocation of activated AR, inhibits DNA binding to androgen response elements, and inhibits recruitment of coactivators, even in the setting of AR overexpression and in prostate cancer cells resistant to antiandrogens. By

contrast with bicalutamide, enzalutamide is a pure antagonist with no detectable agonist effects in LNCaP/AR prostate cells, which overexpress AR. The drug also induces regression of established LNCaP/AR xenograft tumors growing in castrated male mice, a model in which bicalutamide treatment only slows tumor growth.

Clinical Data with Enzalutamide

A phase I/II first in man study was initiated in July 2007 at three sites to assess safety, pharmacokinetics, tolerability, and antitumor activity.[8] Patients with progressive, metastatic CRPC were eligible. The initial dose of enzalutamide was 30 mg daily, with subsequent dose-escalations in cohorts of 3-6 patients to a maximum dose of 600 mg daily. When significant >50% declines in PSA levels were observed in the first six patients, enrollment was expanded by an additional 24 patients (12 pre-chemotherapy, 12 post-chemotherapy) at every dose level starting at 60 mg daily. By 12/2008, 140 patients had been enrolled.

After administration of one dose, the drug was rapidly absorbed, and median time to C_{max} was one hour (range 0.42 minutes – 4 hours). The $t_{1/2}$ was about 1 week (range 3 – 10 days) and was not affected by dose. Full pharmacokinetic profiles were linear and consistent over the dose range study. Plasma concentrations reached steady state after one month of treatment. Once achievement of steady state, the C_{min} in individual patients remained constant for several months, suggesting time-linear pharmacokinetics. Due to slow clearance from plasma, the daily fluctuation in steady-state enzalutamide concentrations were low. The mean C_{max}/C_{min} was 1.2 (range 1.14-1.3) indicating that the average difference between the peak and trough concentrations was ≤ 30%. AR binding was assessed in 22 patients at doses from 60-480 mg daily with FHDT-PET. All patients showed clear reduction of FDHT uptake (range 20-100%).

Fatigue was the most frequently reported AE, with dose-dependent increases of grade 3 fatigue (0% at 150 mg/day, 9% at 240 mg/day, 15% at 360 mg/day, and 20% at 480 mg/day). The dose of 240 mg/day was defined as the maximum tolerated dose. At doses of 240 mg and above, an increasing proportion of patients needed dose reductions for fatigue. Dose reductions were needed in 1 of 29 patients (3%) that received 240 mg/day, 3 of 28 patients (11%) that received 360 mg/day, and 5 of 22 patients (23%) that received 480 mg/day, and 0 of 58 patients that received 30, 60, or 150 mg/day. After dose reductions, the symptoms resolved. Only 1 patient discontinued treatment due to fatigue with an onset coinciding with PSA rise. Overall, the most common mild (grade 2) AEs were fatigue (n = 38, 27.1%), nausea (n = 12, 8.6%), dyspnea (n = 11, 7.9%), anorexia (n = 8, 5.7%), and back pain (n = 8, 5.7%). Fatigue, nausea, and anorexia were the only mild AEs with an increasing incidence as the dose of enzalutamide was increased. None of the grade 2 events required dose modification or the discontinuation of treatment, apart from 1 patient treated at 480 mg/day who had nausea at baseline and stopped therapy after 7 weeks.

Two witnessed seizures occurred in patients receiving doses of 600 and 360 mg/day, and 1 possible seizure occurred at 480 mg/day. Whether enzalutamide was responsible for these seizures is unclear, since both patients who had witnessed seizures were concurrently taking drugs that could contribute to a lowered seizure threshold (olanzapine and prochlorperazine for the patient receiving 600 mg/day; methylphenidate for the patient receiving 360 mg/day). Both patients also had complicated medical problems that could have contributed to their seizures, including hypocalcaemia requiring intravenous calcium, anemia requiring red-cell transfusions, and skull metastases requiring skull radiation. Other causes of treatment discontinuation included rash in 1 patient that received 480 mg/day after 10 days and in 1 patient that received 600 mg/day after 3 days, and a myocardial infarction after 15 weeks of therapy in a patient with a history of diabetes, hypertension, and hypercholesterolemia that received 360 mg/day. All patients recovered without sequelae. No deaths and no other drug-related SAEs were reported.

In regards to efficacy, antitumor effects were noted at all doses including >50% declines in PSA in 78 (56%) patients, response in soft tissue in 13 (22%) of 59 patients, stabilized bone disease in 61 (56%) of 109 patients, and conversion from unfavorable to favorable CTC counts in 25 (49%) of 51 patients. Disease regression was dose dependent between daily doses of 30 mg and 150 mg, however no additional benefit was noted above this threshold.

Based on these results, two placebo-controlled, randomized phase 3 studies (AFFIRM and PREVAIL) were initiated to evaluate the efficacy and safety of enzalutamide in patient with advanced prostate cancer. The AFFIRM study evaluated the safety and efficacy of enzalutamide in 1,199 patients with CRPC after chemotherapy with docetaxel.[9] Patients were randomized in a 2:1 ratio to receive oral enzalutamide at a dose of 160 mg per day or placebo. The primary endpoint was OS. The study was stopped after a planned interim analysis at the time of 520 deaths. The median OS was 18.4 months in the enzalutamide group versus 13.6 months in the placebo group (HR 0.63, 95% CI 0.53-0.75, $p<0.001$). The superiority of enzalutamide over placebo was shown with respect to all secondary endpoints: ≥50% PSA reduction (54% vs. 2%, $p<0.001$), soft-tissue response rate (29% vs. 4%, $p<0.001$), the quality-of-life response rate (43% vs. 18%, $p<0.001$), time to PSA progression (8.3 vs. 3.0 months, $p<0.001$), time to first SRE (16.7 vs. 13.3 months, $p<0.001$).

The rates of AEs (Adverse events) between the enzalutamide and placebo group were similar. The enzalutamide group had a lower incidence of AEs of grade 3 or above (45.3% vs. 53.1%). The median time to first AE was 12.6 months in the enzalutamide group compared to 4.2 months in the placebo group. There was a higher incidence of all grades of fatigue, diarrhea, hot flashes, musculoskeletal pain, and headache in the enzalutamide group compared to placebo. Cardiac disorders were noted in 6% of patients receiving enzalutamide and in 8% of patients receiving placebo. Hypertension was observed in 6.6% of patients in the

enzalutamide group compared to 3.3% in the placebo group. LFT abnormalities were reported as AEs in 1% and 2% of the enzalutamide and placebo group, respectively. Five of the 800 patients in the enzalutamide group (0.6%) were reported to have seizures and no seizures were reported in the placebo group. One case of status epilepticus required medical intervention while the other four seizures were self-limited. There were potentially predisposing factors in several patients, including two patients who had brain metastases, one patient who had inadvertently been administered lidocaine intravenously, and one patient with brain atrophy in the context of heavy alcohol use and initiation of haloperidol. Based on the results of this trial, the FDA approved enzalutamide on 8/31/2012 for the treatment of patients with metastatic CRPC who have previously received docetaxel.

The PREVAIL trial is a double-blinded, randomized, placebo-controlled trial is investigating the effectiveness of enzalutamide in patients with metastatic CRPC who have not yet received chemotherapy. The primary endpoints are OS (Overall survival) and PFS (Progression Free Survival). The trial has reached its target accrual of 1,680 patients.

2.2 Study Disease

Prostate cancer is the most common cancer in men in the United States, with a life time risk of 16%, and the second leading cause of death in this population.[10] In 2012, it is estimated that 241,740 men will be diagnosed with prostate cancer and 28,170 will die of the disease.[10]

ADT (Androgen Deprivation Therapy) is the mainstay of systemic therapy for patients with prostate cancer. In patients with advanced disease, despite initial response rates of 80-90%, nearly all men develop progressive disease within 18-24 months of therapy.[11] Men with CRPC have a poor prognosis with median survival of 16-18 months and fewer than 20% of patients surviving beyond three years.[12]

2.3 Rationale

Recent advances in genomics provide unprecedented opportunities for translational objectives that inform both treatment response and disease biology. Molecular mechanisms of resistance to targeted anti-cancer agents reported in the past few years include secondary mutations in target genes, gene amplification and/or overexpression, generation of alternatively spliced transcripts, production of new fusion genes, mutations in downstream targets and genetic activation of alternate pathways.

The purpose of this study is to elucidate AR related mechanisms of resistance to enzalutamide via assessment of serial CRPC metastasis biopsies. Tumor analysis will include AR sequencing (mutations, splice-variants), AR regulated gene expression, tumor androgen levels, and profiling of enzymes involved in androgen synthesis/metabolism. Additionally, via whole-exome and transcriptome sequencing we

have the opportunity to perform more holistic studies of drug resistance. Additionally, CTCs were measured in Phase I/II trial of enzalutamide and cell numbers were demonstrated to correlate with clinical response. We propose to measure CTCs to assess response to enzalutamide, in addition to analyzing CTCs for mechanisms of androgen resistance.

2.4 Correlative Studies Background

The AR gene is located on chromosome Xq11-12 and is a member of the steroid hormone receptor family.[13] The AR is present in benign prostate epithelial cells as well as in all stages and grades of primary and metastatic prostate cancer. The AR is a 110 kDa ligand-activated transcription factor consisting of 917 amino acids that contain three important domains: 1) a carboxy-terminal LBD which binds androgens, 2) a DBD, and 3) a regulatory NTD. Within the NTD lies the AF-1 and AF-5 domains which contain binding sites for transcriptional coregulators and are essential for AR activity. Under normal conditions, inactive AR is found in the cytoplasm of prostate cells and is stabilized by various HSPs, which expose the LBD and allow ligand binding. Once bound to ligand, a conformational change occurs in the AR, causing dissociation of the HSPs, AR dimerization, and AR migration to the nucleus. Once inside the nucleus, the AR DBD binds to specific AREs on the promoter or enhancer regions of androgen-regulated genes. Transcription is enhanced by the binding of coregulators to AF-1.

Recently, it has become clear that resistance to ADT is mediated by upregulation of AR signaling, despite castrate levels of serum testosterone. Proposed mechanisms of resistance to castration include: 1) increased AR expression leading to AR activation in the presence of low levels of ligand, 2) AR gene mutations leading to promiscuous activation of AR signaling by various ligands, 3) AR splice variants leading to constitutive activation of AR signaling, and 4) increased intra-tumor expression of enzymes involve in steroidogenesis.[14-16]

The AR is expressed at high levels in most cases of CRPC, with the AR gene being amplified in about one third of cases.[17, 18] Mutations in the AR that cause promiscuous activation by ligand have been detected in at least 15-40% of patients after development of resistance.[19] Stranbrough and colleagues profiled the expression signatures of primary and metastatic prostate tumors, including both castration-sensitive and castration-resistant tumors, and found significant increased intratumoral expression of AR and genes converting adrenal androgens to testosterone and DHT in castrate-resistant tumors.[20] Similarly, Montgomery *et al* showed that metastatic prostate cancers from anorchid men express transcripts encoding steroidogenic enzymes and maintain intratumoral androgens at concentrations capable of activating AR target genes and maintaining tumor cell survival.

Altered AR mRNA splicing and synthesis of truncated AR variant proteins lacking the AR LBD is another mechanism that has been postulated to drive overall resistance to

ADT. Although many forms of these splice-variants are maladaptive and lead to a nonfunctioning AR protein, AR variants lacking the LBD but retaining the DBD and NTD are frequently expressed in CRPC metastases and high levels in prostate cancer tissue predict disease progression and shorter survival.[21-24] AR splice-variant mutants can thereby be rendered insensitive to circulating or local hormone levels. Recently, it was shown the AR gene rearrangements expressing both full-length and AR variants are androgen independent and enzalutamide resistant.[25]

3. PARTICIPANT SELECTION

The inclusion and exclusion criteria for enrolling subjects in this study are described in the following sections. If there is a question about the inclusion or exclusion criteria, the investigator should consult with the PI, [redacted] and the DFCI study team before enrolling the subject in the study.

3.1 Eligibility Criteria

Participants must meet the following criteria on screening examination to be eligible to participate in the study:

3.1.1 Be a male ≥ 18 years of age.

3.1.2 Participants must have histologically or cytologically confirmed adenocarcinoma of the prostate without ≥50% neuroendocrine differentiation or small cell histology.

3.1.3 Participants must have progressive disease as defined by either:

- Castrate resistant disease as defined by PCWG.[26] Participants must have a rise in PSA on two successive determinations at least one week apart and PSA levels ≥ 2 ng/ml (only the screening PSA needs to be ≥ 2 ng/ml) and testosterone levels < 50 ng/dL, OR
- Soft tissue progression defined by RECIST 1.1, OR
- Bone disease progression defined by PCWG2 with two or more new lesions on bone scan.

3.1.4 Participants must have a testosterone levels < 50 ng/dL.

3.1.5 CRPC with metastatic disease with at least one site of metastatic disease must be amenable to needle biopsy. Soft tissue biopsy sites include: lymph node or visceral metastases. Bone sites include lumbar vertebrae, pelvic bones and long bones. Excluded sites are thoracic, cervical vertebrae, skull and rib lesions. Biopsy site will be selected with guidance of interventional radiologist determining best site to optimize balance of obtaining useful tissue for analysis and minimizing risk.

3.1.6 Participants without orchiectomy must be maintained on LHRH agonist/antagonist therapy.

3.1.7 Participants may have had any number of previous hormonal therapies (antiandrogens, estrogens, finasteride, dutasteride, ketoconazole, abiraterone) provided these were discontinued ≥ 4 weeks before enrollment. Participants on prednisone 10 mg daily or another equivalent steroid dose are eligible. Participants on inhaled steroids are eligible.

3.1.8 Participants may have had up to two previous cytotoxic therapeutic regimens provided these were discontinued ≥ 4 weeks before enrollment. The use of antineoplastic agents for non-cancer therapy (i.e. colitis, rheumatoid arthritis) may be allowed provided the patient has been on a stable dose without toxicities greater than grade 1.

3.1.9 At least a 4 week interval from previous prostate cancer treatment other than LHRH agonist/antagonist therapy, bisphosphonates, or denosumab to enrollment.

3.1.10 Participants receiving bisphosphonates therapy or denosumab can be maintained on this therapy. If participants have not started bisphosphonates or denosumab, it is recommended that they start treatment after the first biopsy.

3.1.11 ECOG performance status < 2 (Karnofsky >60%, see Appendix A).

3.1.12 Participants must have normal organ and marrow function as defined below:

- WBC ≥ 3,000/mcL
- ANC ≥ 1,500/mcL
- Platelets ≥ 100,000/mcL
- Hemoglobin ≥ 9 g/dL
- Serum albumin ≥ 3.0 g/dL
- AST, ALT, and total bilirubin ≤ 1.5 x Institutional ULN
- Creatinine ≤ 1.5 Institutional ULN or a calculated creatinine clearance **≥** 50 mL/min using the Cockcroft Gault equation
- PTT ≤ 60, INR ≤ 1.5 Institutional ULN unless on warfarin therapy (investigator would need to determine if safe for participant to stop warfarin prior to biopsy)

3.1.13 Have signed an informed consent document indicating that the subjects understands the purpose of and procedures required for the study and are willing to participate in the study.

3.1.14 Be willing/able to adhere to the prohibitions and restrictions specified in this protocol.

3.1.15 Written Authorization for Use and Release of Health and Research Study Information (US sites only) has been obtained.

3.1.16 Able to swallow the study drug whole as a tablet.

3.1.17 Participants 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 during the treatment period and for 1 week after last dose of enzalutamide.

3.2 Exclusion Criteria

Participants who exhibit any of the following conditions at screening will not be eligible for admission into the study.

3.2.1 Uncontrolled illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements within 6 months of enrollment.

3.2.2 Clinically significant heart disease as evidenced by:

- Myocardial infarction within 6 months of enrollment.
- Uncontrolled angina within 6 months of enrollment.
- Congestive heart failure NYHA Class III or IV, or a history of congestive heart failure NYHA Class III or IV in the past, unless a screening ECHO or MUGA within 3 months results in a left ventricular ejection fraction ≥ 45%.
- Clinically significant ventricular arrhythmias.
- History of Mobitz II second degree or third degree heart block without a permanent pacemaker in place.
- Bradycardia as indicated by a heart rate < 50 beats per minute at screening visit.
- Hypotension as indicated by SBP ≤ 85 on 2 consecutive measurements.
- Uncontrolled hypertension as indicated by SBP > 170 mmHg or DBP > 105 mmHg on 2 consecutive measurements at screening visit.

3.2.3 Thromboembolism within 6 months of enrollment.

3.2.4 History of gastrointestinal disorders (medical disorders or extensive surgery) which may interfere with the absorption of the study drug.

3.2.5 History of seizure or any condition that may predispose to seizure (e.g., prior cortical stroke or significant brain trauma, history of loss of consciousness or

transient ischemic attack within 12 months of study entry) or concurrent medication that may predispose to seizure (see Appendix D). Diabetics on a stable dose of insulin or antihyperglycemic regimen are allowed if they have had no prior seizures and no history of loss of consciousness due to hypoglycemia.

3.2.6 Individuals with a history of a different malignancy and are on treatment for the malignancy or the malignancy is documented on screening or other imaging are ineligible. Individuals with a history of other malignancies are eligible if they are deemed by the investigator to be at low risk for recurrence of that malignancy.

3.2.7 Known brain metastasis. Participants with brain metastasis can only be included if they were treated > 4 week prior to enrollment with radiation and the effects of treatment have resolved. Subjects with treated brain metastases must have a post-treatment brain MRI showing no further progression of prior lesions and no new metastatic lesions. Subjects will be ineligible if they have any ongoing symptoms from brain metastases or if there continues to be radiographic evidence of cerebral edema.

3.2.8 Have known allergies, hypersensitivity, or intolerance to enzalutamide or their excipients.

3.2.9 Surgery or local prostatic intervention within 30 days of the first dose. In addition, any clinically relevant issues from the surgery must have resolved prior to enrollment.

3.2.10 Major surgery or radiation therapy within 4 weeks of enrollment.

3.2.11 Radium-223, strontium-89, or samarium-153 therapy within 4 weeks of enrollment.

3.2.12 Radiotherapy, chemotherapy or immunotherapy within 4 weeks, or single fraction of palliative radiotherapy within 14 days of administration of enrollment.

3.2.13 Prior treatment with enzalutamide.

3.2.14 Current enrollment in an investigational drug or device study or participation in such a study within 30 days of enrollment.

3.2.15 Concomitant use of medications that may alter pharmacokinetics of enzalutamide. See section 5.3.

3.2.16 Any acute toxicities due to prior chemotherapy and/or radiotherapy that have not resolved to a NCI CTCAE (version 4) grade of ≤ 1. Chemotherapy induced alopecia and grade 2 peripheral neuropathy are allowed.

3.2.17 Condition or situation which, in the investigator's opinion, may put the participant at significant risk, may confound the study results, or may interfere significantly with participant's participation in the study.

3.2.18 Individuals not willing to comply with the procedural requirements of this protocol.

3.2.19 HIV-positive individuals on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with enzalutamide. Appropriate studies will be undertaken in participants receiving combination antiretroviral therapy when indicated.

3.3 Inclusion of Minorities and Other Underrepresented Populations

Every effort will be made to include men from minority populations. The enrollment of minority men will reflect the proportion of minority participants at the sites participating in the trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC and DF/PCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. Registration cancellations must be made in OnCore as soon as possible.

4.2 Registration Process for DF/HCC and DF/PCC Institutions

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

4.3 General Guidelines for Other Participating Institutions

Eligible participants will be entered on study centrally at DFCI by the Study Coordinator. All sites should call the Study Coordinator at [redacted] to verify treatment availability. The required forms will be provided to all participating institutions by the DFCI study coordination.

Following registration, participants should begin protocol treatment within 5 days. Issues that would cause treatment delays should be discussed with the Overall PI. If a participant does not receive protocol therapy following registration, the participant's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

4.4 Registration Process for Other Participating Institutions

To register a participant, the documents listed in Appendix B should be completed by the research nurse or data manager and faxed to [redacted] or emailed to the DFCI study coordinator (see section 3.7.1 of the DSMP (Appendix E).

The research nurse or data manager at the participating site will then call or e-mail the Study Coordinator to verify eligibility. To complete the registration process, the Coordinator will follow DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) and register the participant on the protocol. The coordinator will fax or e-mail the participant study number, and if applicable the dose treatment level, to the participating site.

5. TREATMENT PLAN

All protocol required therapy will be prescribed by prescription and taken by the participant on an outpatient basis. A baseline metastasis biopsy will be done prior to starting protocol therapy.

Participants will be treated with four 40 mg capsules (160 mg) once daily of enzalutamide taken orally. All participants without orchiectomy will be maintained on LHRH agonist/antagonist therapy. Participants will be evaluated clinically and with laboratory studies on day 1 of every 28 day cycle. Participants will maintain a drug diary from time of initiation of study treatment to time of discontinuation from the study (Appendix C). Participants will be continued on therapy until evidence of symptomatic or radiographic progression or taken off study for another reason.

A metastasis biopsy will be performed while the participant is still on enzalutamide at the time of progression. For DFCI participants, if the biopsy site (baseline and progression) is a pelvic bone both an aspirate and core biopsy will be obtained. After progression biopsy is performed, protocol therapy will be discontinued. Participants who stop protocol therapy before receiving four cycles of enzalutamide will not be asked to undergo a second biopsy. The tumor biopsy at the end of study is otherwise mandatory and can be done +/- 14 days of determination of progression. Situation where the participant stops protocol therapy for reasons other than progression after receiving 4 cycles of enzalutamide should be discussed with the PI if it is felt that biopsy would not represent progressive disease. For DFCI participants, quantitative SPECT/CT imaging substudy will be performed in addition to standard bone scanning at baseline and at the time of the first two follow up bone scans.

Expected toxicities and potential risks as well as dose modifications for enzalutamide are described in Section 6 (Expected Toxicities and Dosing Delays/Dose Modifications). No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Treatment Description					
Agent	**Pre-medications; Precautions**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Enzalutamide	Can be taken with or without food. The capsules should be swallowed whole with water. Do not chew, dissolve, or open capsules.	160 mg	Oral	Once daily	28 days (4 weeks)

5.1 Pre-treatment Criteria

5.1.1 Cycle 1, Day 1: The following parameters must be met:

- WBC ≥ 3,000/mcL
- ANC ≥ 1,500/mcL
- Hemoglobin ≥ 9 g/dL
- AST/ALT ≤ 1.5 x Institutional ULN
- Total bilirubin ≤ 1.5 x Institutional ULN

If these parameters are not met, the participant can be evaluated on a weekly basis.

Agent Administration

5.2.1 Enzalutamide

- Administration: 160 mg (oral) once daily. Treatment will be continued until evidence of symptomatic or radiographic progression or the participant is taken off the study for another reason.

- Dosing: 160 mg (oral) taken once daily as four 40 mg capsules. Possible dose modifications are outlined in Section 6.

- Vital Signs: Vital signs including blood pressure, pulse, weight and temperature should be taken on day 1 of each cycle, and at the time of any symptom evaluation.

- Compliance: Enzalutamide can be taken with or without food. Enzalutamide may be taken at any time during the day, but should be taken at the same time each day. A missed dose should be taken as soon it is remembered. If twelve or more hours have lapsed since a missed dose, participants should not take the missed dose; rather the participant should resume dosing at the next scheduled dose. Participants should not double up or take more than one dose of enzalutamide per day. Vomited doses should not be made up. If doing compliance is not 100% in the absence of toxicity, the participant should be re-instructed regarding the proper dosing procedures and continue in the protocol. Participants will be asked to record actual dosing in a drug diary (Appendix C).

5.3 General Concomitant Medications and Supportive Care Guidelines

Supportive Care Radiation
Palliative radiation to any bone lesion is allowed if the patient has otherwise stable disease. Local palliative radiation to the area of the prostate or pelvis may also be allowed if the patient has otherwise stable disease.

Drugs that Inhibit or Induce CYP2C8
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 composite AUC of enzalutamide plus N-desmethyl enzalutamide by 2.2-fold with minimum effect on C_{max}. Therefore, co-administration of enzalutamide with strong CYP2C8 inhibitors should be avoided as they would require a dose reduction of enzalutamide, which will not be permitted at study drug initiation. If co-administration of enzalutamide with a strong CYP2C8 inhibitor cannot be avoided, the dose of enzalutamide should be reduced to 80 mg once daily.

The effects of CYP2C8 inducers on the pharmacokinetics of enzalutamide have not been evaluated *in vivo*. Co-administration of enzalutamide with strong or moderate CYP2C8 inducers (i.e. rifampin) may alter the plasma exposure of enzalutamide and should be avoided.

Drugs that Inhibit or Induce CYP3A4
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 $AUC_{0-\infty}$ of enzalutamide plus N-desmethyl enzalutamide by 1.3-fold with no effect on $C_{max.}$

The effects of CYP3A4 inducers on the pharmacokinetics of enzalutamide have not been evaluated *in vivo*. Co-administration of enzalutamide with strong CYP2C8 inducers (i.e. carbamazepine, phenobarbital, phenytoin, rifabutin, rifamin, rifapentine) may decrease the plasma exposure of enzalutamide and should be avoided. Moderate CYP3A4 inducers (i.e. bosentan, efavirenz, etravirine, modafinil, nafcillin) and St. John's Wort may also reduce the plasma exposure of enzalutamide and should be avoided.

Effects of Enzalutamide on Drug Metabolizing Enzymes
In an *in vivo* phenotypic cocktail drug-drug interaction trial in patients with CRPC, a single oral dose of the CYP probe cocktail (for CYP2C8, CYP2C9, CYP2C19, CYP3A4) was administered before and concomitantly with enzalutamide (following at least 55 days of dosing at 160 mg daily). Results showed that *in vivo*, at steady state, enzalutamide reduced the plasma exposure to midazolam (CYP3A4 substrate), warfarin (CYP2C9 substrate), and omeprazole (CYP2C19 substrate). Thus, enzalutamide is a strong CYP3A4 inducers and a moderate CYP2C9 and CYP2C19 inducer. Concomitant use of enzalutamide with narrow therapeutic index drugs that are metabolized by CYP3A4 (i.e. alfentanil, cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus and tacrolimus), CYP2C9 (i.e. phenytoin, warfarin) and CYP2C19 (i.e. S-mephenytoin) should be avoided, as enzalutamide may decrease their exposure. If co-administration with warfarin cannot be avoided, additional INR monitoring is warranted. In this study, enzalutamide did not cause clinically meaningful changes in exposure to the CYP2C8 substrate (pioglitazone).

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. *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.

Class	**Example Drugs**	**Recommendation**
Strong CYP2C8 Inhibitors	Gemfibrozil	If co-administration with gemfibrozil cannot be avoid, reduce enzalutamide dose
Strong CYP2C8 Inducers	Rifampin	Concomitant use ineligible
Strong CYP3A4 Inhibitors	Itraconazole	No initial dose adjustment
Strong/Moderate CYP3A4 Inducers	Carbamazepine, phenobarbital, phenytoin, rifabutin, rifamin, rifapentine, osentan, efavirenz, etravirine, modafinil, nafcillin, and St. John's Wort	Concomitant use ineligible
CYP3A4 Substrate	Alfentanil, cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus and tacrolimus	Avoid concomitant use of substrates with a narrow therapeutic index
CYP2C9 Substrate	Phenytoin, warfarin	Avoid concomitant use of substrates with a narrow therapeutic index
CYP2C19 Substrate	S-mephenytoin	Avoid concomitant use of substrates with a narrow therapeutic index
CYP2C8 Substrate	Pioglitazone	No dose adjustment

If at any time an investigator suspects a drug-drug interaction due to enzalutamide, the PI, [redacted] should be contacted at [redacted] or paged at [redacted] Additional information is provided in the enzalutamide Investigator's Brochure.

5.4 Duration of Therapy

Duration of therapy will depend on individual response, evidence of disease progression, and tolerance. In the absence of treatment delays due to AEs, treatment may continue until one of the following criteria:

- Symptomatic or radiographic disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable AE(s)
- Treatment emergent seizure
- Participant decided to withdraw from the study
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the opinion of the treated investigator.

Protocol therapy may be held for up to six weeks in the event of an AE and the participant may be restarted on therapy when the toxicity has resolved to ≤ grade 1, In the event of therapy being held for more than six weeks, it is recommended that the participant come off protocol however the treating physician may obtain permission to continue on the protocol with permission of the PI, [redacted] if the treating physician feels it is in the participant's best interest.

5.5 Duration of Follow-Up

Participants will be followed for up to 5 years post-study discontinuation or until death, whichever comes first. Participant follow up visits will be at the research clinic for the first 2 years of follow-up. Following the first 2 years, participant follow-up can be done via medical record review and/or by phone. Participants will be followed for subsequent lines of therapy, including line of agent, name of agent, and PSA kinetics following study drug discontinuation and correlate with response to study drug. This information will be updated every 6 months. The research team will collect this information during patient clinic visits, by phone, or via medical record review.

5.6 Criteria for Removal from Study

Participants will be removed from study when any of the criteria listed in Section 5.4 applies. The reason for study removal and the date the participant was removed must be documented in the study-specific CRF. Alternative care options will be discussed with the participant.

Participants will be removed from treatment at the time of unacceptable AEs but will remain on study (i.e. enrolled on the protocol) until resolution of any AEs.

In the event of unusual or life-threatening complications, participating investigators must immediately notify the PI, [redacted] at [redacted] or page [redacted]

6. EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

The starting dose of enzalutamide will be 160 mg daily. In the presence of any enzalutamide related toxicities > grade 2 (NCI CTCAE version 4), during treatment with enzalutamide at the starting dose level, the dose of enzalutamide will be decreased to 120 mg daily in the subsequent dose level. Two levels of dose de-escalation are planned for the study. If > grade 2 toxicity occurs at dose level -2, participants will be removed from the protocol.

Dose Level	Enzalutamide
Starting dose level	160 mg orally once daily
-1	120 mg orally once daily
-2	80 mg orally once daily

Toxicity assessments will be done using the NCI Common Terminology Criteria for Adverse Events (CTCAE) (version 4) which is identified and located on the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

If possible, symptoms should be managed symptomatically. In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).

All AEs experienced by participants will be collected from the time of the first dose of study treatment, through the study and until the final study visit. Participants continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

6.1 Anticipated Toxicities

A list of the AEs and potential risks associated with the agents administered in this study appear below and will determine whether dose delays and modifications will be made or whether the event requires expedited reporting in addition to routine reporting.

6.1.1 AEs for Enzalutamide

In the randomized clinical trial in patients with metastatic CRPC who had previously received docetaxel, patients received enzalutamide 160 mg orally once daily (N = 800) or placebo (N = 399). The median duration of treatment was 8.3 months with enzalutamide and 3.0 months with placebo. All patients continued ADT. Patients were allowed, but not required, to take glucocorticoids. During the trial, 48% of patients on the enzalutamide arm and 46% of patients on the placebo arm received glucocorticoids. All AEs and laboratory abnormalities were graded using NCI CTCAE version 4.

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, Institutional ULN 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 AEs 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.

Seizures
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 regarding re-administering enzalutamide to patients who experienced seizures. See Appendix D for representative medications that may predispose to seizure.

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.

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 SAEss were reported in approximately 6% of the patients on both treatment arms.

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.

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.

6.2 Toxicity Management/Dose Modifications/Delays

Enzalutamide is generally well-tolerated based on phase III data. Instructions on management of enzalutamide related toxicities > grade 2 (NCI CTCAE version 4) are detailed in section 6.0. Evidence of seizure will results in cessation of treatment with enzalutamide and withdrawal from the study. Management of other toxicities is detailed below.

- Grade 1-2 Toxicities: Management per investigator. No study treatment dose reduction indicated.

- Grade 3 of Higher Toxicities: Hold enzalutamide. Enzalutamide can be held for a maximum of 6 weeks. Hold will not affect assessment schedule. When toxicity resolves to ≤ grade 1, resume at first dose modification level (120 mg daily). Prophylactic medications should be considered. If toxicity recurs, hold study medication and adjust or add medications to mitigate the toxicity. When recurrent toxicity has resolved to ≤ grade 1, resume enzalutamide at 80 mg daily. If grade 3 toxicity recurs, the participant will be discontinued from the study.

Dose may NOT be re-escalated after it has been reduced.

7. DRUG FORMULATION AND ADMINISTRATION

7.1 Enzalutamide

Refer to the package insert for enzalutamide information.

7.1.1 Description

Enzalutamide is an androgen receptor inhibitor. The chemical name is 4-{3-[4-cyano-3-(trifluoromethyl)phenyl]-5,5dimethyl-4-oxo-2-sulfanylideneimidazolidin-1-yl}-2-fluoro-N-methylbenzamide.
The molecular weight is 464.44 and molecular formula is $C_{21}H_{16}F_4N_4O_2S$.

7.1.2 Mechanism of Action

Enzalutamide is an androgen receptor inhibitor that acts on different steps in the androgen receptor signaling pathway. Enzalutamide has been shown to competitively inhibit androgen binding to androgen receptors and inhibit androgen receptor nuclear translocation and interaction with DNA. A major metabolite, N-desmethyl enzalutamide, exhibited similar *in vitro* activity to enzalutamide. Enzalutamide decreased proliferation and induced cell death of prostate cancer cells *in vitro*, and decreased tumor volume in a mouse prostate cancer xenograft model.

7.1.3 Pharmacokinetics

The pharmacokinetics of enzalutamide and its major active metabolite (N-desmethyl enzalutamide) were evaluated in patients with metastatic CRPCand healthy male volunteers. The plasma enzalutamide pharmacokinetics are adequately described by a linear two-compartment model with first-order absorption.

Absorption

Following oral administration (enzalutamide 160 mg daily) in patients with metastatic CRPC, the median time to reach C_{max} is 1 hour (range 0.5 to 3 hours). At steady state, the plasma mean C_{max} values for enzalutamide and N-desmethyl enzalutamide are 16.6 µg/mL (23% CV) and 12.7 µg/mL (30% CV), respectively, and the plasma mean predose trough values are 11.4 µg/mL (26% CV) and 13.0 µg/mL (30% CV), respectively.

With the daily dosing regimen, enzalutamide steady state is achieved by Day 28, and enzalutamide accumulates approximately 8.3-fold relative to a single dose. Daily fluctuations in enzalutamide plasma concentrations are low (mean peak-to-trough ratio of 1.25). At steady state, enzalutamide showed approximately dose proportional pharmacokinetics over the daily dose range of 30 to 360 mg.

A single 160 mg oral dose of enzalutamide was administered to healthy volunteers with a high-fat meal or in the fasted condition. A high-fat meal did not alter the AUC to enzalutamide or N-desmethyl enzalutamide.

Distribution and Protein Binding
The mean apparent volume of distribution of enzalutamide in patients after a single oral dose is 110 L (29% CV). Enzalutamide is 97% to 98% bound to plasma proteins, primarily albumin. N-desmethyl enzalutamide is 95% bound to plasma proteins.

Metabolism
Following single oral administration of ^{14}C-enzalutamide 160 mg, plasma samples were analyzed for enzalutamide and its metabolites up to 77 days post dose. Enzalutamide, N-desmethyl enzalutamide, and a major inactive carboxylic acid metabolite accounted for 88% of the ^{14}C-radioactivity in plasma, representing 30%, 49%, and 10%, respectively, of the total ^{14}C-AUC$_{0-\infty}$.

In vitro, human CYP2C8 and CYP3A4 are responsible for the metabolism of enzalutamide. Based on *in vivo* and *in vitro* data, CYP2C8 is primarily responsible for the formation of the active metabolite (N-desmethyl enzalutamide).

Elimination
Enzalutamide is primarily eliminated by hepatic metabolism. Following single oral administration of ^{14}C-enzalutamide 160 mg, 85% of the radioactivity is recovered by 77 days post dose: 71% is recovered in urine (including only trace amounts of enzalutamide and N-desmethyl enzalutamide), and 14% is recovered in feces (0.4% of dose as unchanged enzalutamide and 1% as N-desmethyl enzalutamide).

The mean apparent clearance of enzalutamide in patients after a single oral dose is 0.56 L/h (range 0.33 to 1.02 L/h).

The mean terminal $t_{1/2}$ for enzalutamide in patients after a single oral dose is 5.8 days (range 2.8 to 10.2 days). Following a single 160 mg oral dose of enzalutamide in healthy volunteers, the mean terminal $t_{1/2}$ for N-desmethyl enzalutamide is approximately 7.8 to 8.6 days.

7.1.4 Form
Enzalutamide is a white crystalline non-hygroscopic solid. It is practically insoluble in water. Enzalutamide is provided as liquid-filled soft gelatin capsules for oral administration. Each capsule contains 40 mg of enzalutamide as a solution in caprylocaproyl polyoxylglycerides. The inactive ingredients are caprylocaproyl polyoxylglycerides, butylated hydroxyanisole, butylated hydroxytoluene, gelatin, sorbitol sorbitan solution, glycerin, purified water, titanium dioxide, and black iron oxide.

7.1.5 Storage and Stability
Pharmacy Storage Requirements
The study treatment will be stored in a secure area and administered only to participants entered into the clinical study in accordance with the conditions specified in this protocol. Bottles of study treatment should be stored at a room

temperature between 20º-25º C in a dry place and kept with container tightly closed.

Storage Requirements for the Participant
Bottles of study treatment should be stored at room temperature with the cap kept on tightly and should not be refrigerated. Participants should be advised to keep all medications out of the reach and out of sight of children.

Enzalutamide should not be handled by pregnant women.

7.1.6 Compatibility
Treatment with enzalutamide will be administered via oral capsules. Enzalutamide is the only treatment agent which will be administered on the protocol.

7.1.7 Handling
There are no specific instructions for handling enzalutamide. Study treatment must only be dispensed by a Pharmacist or medically qualified staff. Study treatment is to be dispensed only to participants enrolled in this study. Once the study treatment is prepared for a participant, it can only be administered to that participant.

7.1.8 Availability
Enzalutamide capsules will be provided to each site. Participants will be provided with a 30-day supply to allow for visits to occur every 28 days with a ± 3 day window. Information presented on the labels for investigative product will comply with applicable local regulations. Site pharmacist will dispense the study treatment to each participant in accordance with this protocol under the guidelines of the site's dispensation standard operating procedure. The agentwill be provided from commercial supply and will be provided free-of-charge.

7.1.9 Ordering
Enzalutamide is to be ordered from Astellas Pharmaecuticals or their designee. A drug order form as well as instructions on how to order study drug will distributed to study sites prior to site activation.

7.1.10 Accountability
Accountability for study treatment is the responsibility of the investigator.

The study site must maintain accurate records demonstrating dates and amount of study treatment (enzalutamide) received, to whom dispensed (participant by participant accounting), and accounts of any study treatment accidentally or deliberately destroyed. At the end of the study, reconciliation must be made between the amount of study treatment supplied, dispensed, and subsequently destroyed.

At the time of delivery of study treatment to the site, the investigator, designee, or Pharmacist (where appropriate) will confirm that the supplies for the study have been received. This following information will be confirmed: lot numbers, quantities shipped/delivered, and date of receipt.

7.1.11 Destruction and Return

Drug should be destroyed at the site, after the sponsor– investigator approves the drug destruction policy at the site. Destruction will be documented in the Drug Accountability Record Form.

8. CORRELATIVE/SPECIAL STUDIES

Correlative studies will include analysis of prostate tumor from serial biopsies for AR modifications including AR sequence-mutations/splice variants, AR regulated gene expression, tumor androgen levels, profiling of enzymes involved in androgen synthesis and metabolism, and whole- exome and transcriptome sequencing. In addition, as an exploratory analysis we will analyze CTCs for mechanisms of AR resistance to include AR nuclear localization, AR sequencing, AR splice variant analysis and whole-exome and transcriptome sequencing. Lastly, we will analyze circulating tumor DNA for mechanisms of AR resistance to include AR sequencing and whole- exome and transcriptome sequencing. In addition, serum androgens will be measured. The correlative studies are required. A Laboratory Manual with instructions on sample collection and processing will be provided to study sites.

CTC research samples will be sent to the [redacted] at the University of Wisconsin for analysis purposes.

8.1 Statistical Data Analysis

Determination of biological and molecular endpoints to analyze possible AR related mechanisms of resistance to enzalutamide in serial tumor biopsies will occur as follows:

- AR sequencing
  - Mutations – continuous variable from microarray data
  - TMPRSS2:ERG translocation status – categorical variable from PT PCR (yes vs. no)
  - AR splice variants – categorical variable from RT PCR (native vs. alternative)
- AR regulated gene expression – continuous variable from microarray data
- Tumor androgen levels – continuous variable from mass spectroscopy data
- Profiling of enzymes involved in androgen synthesis/metabolism – continuous variable from microarray data

- Whole-exome and transcriptome sequencing – continuous variable from sequencing analysis

Continuous data will be evaluated by calculating mean percent change in level. Categorical data will be evaluated by yes/no change in status. Ordinal/count number data will be evaluated by mean percent change in total count.

9. STUDY CALENDAR

	Pre- Study[a]	Day 1 of Each Cycle[b] (+/- 3 days)	Every 12 weeks (+/- 1 week)	End of Treatment Visit[q]	Follow Up Visits[r] (Every 6 months +/- 1 month)
Informed Consent	X				
History and Physical[c]	X	X		X	
ECOG Performance Status	X	X		X	
Vital Signs[d]	X	X		X	
Hematology[e]	X	X		X	
Serum Chemistry[f]	X	X		X	
Liver Function Test[g]	X	X		X	
Coagulation Factors[h]	X				
EKG	X				
Serum Testosterone	X				
Research Labs[i]	X	X	X	X	
PSA[j]	X	X		X	X
CT/MRI[k]	X		X		
Bone Scan[l]	X		X		
Distribution[m]		X			
Compliance Assessment		X			

Prior and Concomitant Medications	X	X			
Adverse Events[n]	X	X		X	
Tumor Biopsy[o]	X			X[p]	

a: Baseline evaluations are to be conducted within 30 days prior to registration. Scans must be done within 60 days prior to registration. All baseline screening should be done prior to registration.
b: A cycle will be defined as 28 days.
c: Physical examination should include general description of participant, head, eyes, ears, nose, and throat, chest, abdominal, extremities, neurologic, skin, and lymph node examination. Any other evaluation is up to the discretion of the practitioner. It will not be considered a violation if the exam is not described as outlined here.
d: Vital signs included upright blood pressure, heart rate, respiratory rate, body temperature and weight.
e: Hematology testing to include WBC, ANC, hemoglobin, and platelet count.
f: Serum chemistry to include sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, albumin, magnesium.
g: LFTs to include AST, ALT, alkaline phosphatase, total bilirubin and direct bilirubin.
h: Coagulation factors to include PT, PTT, and INR.
i: Research labs to include a blood sample for germline DNA/RNA analysis, serum hormones (to include DHEA, DHEA-S, androstenedione, testosterone, DHT), a blood sample for circulating tumor DNA analysis, a blood sample for CTC analysis. Blood for germline DNA/RNA will be collected at pre-study visit. Hormone levels will be collected at pre-study visit, every 12 weeks on study, at off-study. CTCs will be collected at pre-study visit, Day 1 of each cycle, and off study. Pre-study and off study CTC samples will be shipped overnight. CTCs collected on Day 1 of each cycle will be processed (buffy coat) and stored for delivery. Circulating tumor DNA will be collected at pre-study visit, every 12 weeks on study, at off-study.
j: If a digital rectal examination is performed, PSA must be sampled prior to the examination. For patients enrolling for PSA progression, this is defined as a rise in PSA on two successive determinations at least one week apart and PSA levels $\geq$ 2 ng/ml (only the screening PSA needs to be $\geq$ 2 ng/ml).
k: If baseline CT chest does not demonstrate prostate cancer, do not need to be repeated on study unless the investigator suspects new findings based on clinical signs/symptoms.
l: For DFCI participants only, one bed position of quantitative SPECT/CT will be performed as an imaging substudy concurrently with the standard of care bone scans at baseline and at the time of the first two follow up standard of care bone scans. The field of view will be determined by the nuclear medicine physician depending on the location of the lesions on the planar images.
m: Study agent is enzalutamide. Subjects with be continued on LHRH agonist/antagonist as prescribed. Subjects will begin taking study agent on Day 1/Cycle 1.
n: AEs should be collected from the date informed consent is signed until 30 days after discontinuation from the study.
o: Tumor biopsy must be performed prior to starting study medication. Participants will need to be screening and registered before having a biopsy. Friday should be avoided due to difficult of processing bone biopsies on the weekend. For DFCI participants, if the biopsy site (baseline and progression) is a pelvic bone both an aspirate and core biopsy will be obtained.
p: Participants who stop the protocol therapy before receiving four cycles of enzalutamide will not be asked to undergo the second biopsy. The tumor biopsy at the end of the study can be done +/- 14 days of the determination of progression. Enzalutamide will be discontinued following completion of the second biopsy. Situations where the participant stops protocol therapy for reasons other than progression after receiving four cycles of enzalutamide should be discussed with the PI if it felt that the biopsy would not represent progressive disease.
q: The End of Treatment Visit will be the visit at which it is determined that the patient will no longer be taking treatment given disease progression, toxicity, or other reason.
r: Follow up Visits: Participants will be followed for up to 5 years post-study discontinuation or until death, whichever comes first. Participant follow up visits will be at the research clinic for the first 2 years of follow-up. Following the first 2 years, participant follow-up can be done via medical record review and/or by phone. Participants will be followed for subsequent lines of therapy, including line of agent, name of agent, and PSA kinetics following study drug discontinuation and correlate with response to study drug. This information will be updated every 6 months. The research team will collect this information during patient clinic visits, by phone, or via medical record review.

10. MEASUREMENT OF EFFECT

Although response is not the primary endpoint of this trial, participants with measurable disease will be assessed by RECIST (1.1) for soft tissue progression, PCWG-2 (2008) for bone disease progression, bone scan and PSA criteria. For the purposes of this study, participants will be evaluated every four weeks by PSA and every 12 weeks by CT and bone scan. Participants should not be taken off treatment until documented symptomatic progression or progression by imaging.

The following will be used to determine progression in this study:

- PSA Progression: For those who experience a decline on PSA from baseline, PSA progression will be defined as an increase in PSA that is ≥ 25% and ≥ 2 ng/mL above nadir which is confirmed by a second value three or more weeks later. For participants who do not experience a decline from baseline, PSA progression will be defined as PSA ≥ 25% and ≥ 2 ng/mL after 12 weeks.

- Radiographic Disease Progression: For bone disease, radiographic progression will be defined as the appearance of ≥ 2 new lesions on bone scan. For soft tissue/lymph node disease, radiographic progression will be defined using RECIST 1.1 criteria. PCWG-2 (2008) will be used to define bone disease progression.

- If any of the following criteria that are also described in Section 5.4 are met:
  - Symptomatic or radiographic disease progression
  - Intercurrent illness that prevents further administration of treatment
  - Unacceptable AE(s)
  - Treatment emergent seizure
  - Participant decided to withdraw from the study
  - General or specific changes in the participant's condition render the participant unacceptable for further treatment in the opinion of the treated investigator.

10.1 Antitumor Effect– Solid Tumors

Response and progression will be evaluated in this study using the new international criteria proposed by the RECIST 1.1.

10.1.1 Definitions

Evaluable for Target Disease Response. Only those participants 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 target disease response. These participants will have their response classified according to the definitions stated below. Note: Participants who exhibit objective disease progression or die prior to the end of cycle 1 will also be considered evaluable.

Evaluable for Non-Target Disease Response. Participants 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. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression or die prior to the end of cycle 1 will also be considered evaluable.)

10.1.2 Disease Parameters

Measurable disease. Measurable disease is the presence of at least one (1) lesion that can be accurately measured in at least one dimension with longest diameter ≥ 20 millimeters (mm) using conventional techniques (CT, MRI, x-ray) or ≥10 mm with spiral CT scan. Measurable lesions must be at least 2 times the slice thickness in mm. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters). Ultrasound cannot be used to measure lesions. A lesion in a previously irradiated area is not eligible for measurable disease unless there is objective evidence of progression of the lesion prior to study enrollment. Lesions in previously irradiated areas must be clearly identified as such.

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 < 15mm short axis, are considered non-measurable disease. Leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses identified by physical exam that are not measurable by reproducible imaging techniques, and cystic lesions are all 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. Lesions must be accurately measured in 1 dimension with a minimum size of 10 mm by CT or MRI (slice thickness no greater than 5 mm), 20 mm by chest x-ray. Nodes must have a short axis ≥ 15 mm. The short axis should be included in the sum of the lesions in the calculation of response. Nodes that shrink to < 10 mm are considered normal. Target lesions should be selected on the basis of their size, be representative of all the involved organs, and should be lesions that can be followed with reproducible repeated measurements.

Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be considered target lesions if the soft tissue component meets the definition of measurability as defined above. Cystic lesions thought to represent cystic metastases can be considered as target lesions. However, if non-cystic lesions are present, these are preferred for selection as target lesions. Lesions in previously irradiated areas or areas subject to other loco-regional therapy are usually not considered measurable unless there has been demonstrated progression of that lesion.

Non-target lesions. All other lesions, including small lesions < 10 mm or pathological lymph nodes measuring ≥ 10 mm to < 15 mm in short axis, as well as truly non-measurable lesions, which include leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses identified by physical exam that are not measurable by reproducible imaging techniques.

10.1.3 Methods for Evaluation of Disease

All measurements should be taken and recorded in metric notation, using a ruler, calipers, or digital measurement tool. 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 when both methods have been used to assess the anti-tumor effect of a treatment.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥ 10 mm in diameter as assessed using calipers. For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung; however, CT is preferable.

Conventional CT and MRI. These techniques should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

Tumor markers. Tumor markers alone cannot be used to assess response. The following parameters will be recorded on a monthly basis:

- PSA decline will be measuring according to revised PCWG-2 (2008) criteria.[26]
- PSA changes will be recorded on all participants.
- TTPP will be based on revised PCWG-2 (2008) criteria.
- The maximal decline in PSA for each participant will be recorded for each participant.
- The date of the maximal PSA decline (nadir date) will be recorded for each participant, as will the duration from the start of therapy to the nadir PSA.

10.1.4 Response Criteria

10.1.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph node 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 with at least a 5 mm absolute increase in the sum of all lesions. The appearance of one or more new lesions* denotes disease 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.

Unknown (UN): Assessment of target lesions cannot be made due to insufficient or unevaluable data. In this case, a concise explanation must be given.

Note: If tumor response data is missing for target lesions, the overall assessment must be UN unless there is new disease that would result in an overall assessment of PD. However, if there is missing or unevaluable data for non-target lesions, but data is available for all target lesions, the overall response for that time point will be assigned based on the sum LD of all target lesions. Additionally, the assessment of CR cannot be made if there is missing or unevaluable data for non-target lesions. In this case, the overall assessment would be PR.

***Definition of New Lesion**: The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (ex: new bone lesions may be healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size, etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

10.1.4.2 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 Institutional ULN 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 lesions 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.

When the participant also has measurable disease, to achieve 'unequivocal progression' on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest 'increase' in the size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare. When the participant has only non- measurable disease, worsening in non-target disease cannot be easily quantified. A useful test that can be applied when assessing non-targets for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease: i.e. an increase in tumor burden representing an additional 73% increase in 'volume'

(which is equivalent to a 20% increase diameter in measurable lesions), an increase in pleural effusion from 'trace' to 'large', an increase in lymphangitic disease from 'localized' to 'widespread'.

Unknown (UN): Assessment of non-target lesions cannot be made due to insufficient or unevaluable data. In this case, a concise explanation must be given.

***Definition of New Lesion**: The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (ex: new bone lesions may be healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size, etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

10.1.4.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The participant's best response assignment will depend on the achievement of both measurement and confirmation criteria.

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response for 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	

* In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression. Note: Participants 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 Participants 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 an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised.		

10.1.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrence or progressive disease (PD) is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started, or death due to any cause. Participants without events reported are censored at the last disease evaluation.

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent or progressive disease is objectively documented, or death due to any cause. Participants without events reported are censored at the last disease evaluation.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

10.1.6 Time to Progression

Time to Progression: Time to Progression (TTP) is defined as the time from registration to progression, or censored at the date of last disease evaluation for those without progression reported.

10.1.7 Response Review

Central review of the radiology assessments is planned using tumor metrics core at the DFCI.

10.2 Other Response Parameters

Descriptive statistics (including mean, standard deviation (SD), minimum, maximum, median, interquartile range) will be provided on levels of serum hormones and changes from baseline.

10.2.1 SPECT/CT

In an imaging substudy, patients enrolled at DFCI only will undergo quantitative SPECT/CT as part of the standard of care bone scans at baseline, and at the time of the first two follow up bone scans. The field of view will be determined by the nuclear medicine physician depending on the location of the lesions on the planar images. The imaging parameters for SPECT/CT will follow DFCI institutional policies for SPECT/CT bone scanning. xSPECT Quant software will be used to determine activity concentration on quantitative SPECT/CT in target bone lesions before and after therapy. Descriptive statistics (including mean, standard deviation (SD), minimum, maximum, median, interquartile range) will be provided on activity concentration of ^{99m}Tc-MDP on quantitative SPECT/CT. The data collected from the imaging substudy is exploratory and not used as part of decision making for the response assessment.

11. ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Definitions

11.1.1 Adverse Event (AE)

An AE is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study.

Abnormal laboratory values or diagnostic test results constitute AEs only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

11.1.2 Serious adverse event (SAE)

A SAE is any adverse event, occurring at any dose and regardless of causality that:

- Results in death
- Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires or prolongs inpatient hospitalization (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly or birth defect; or
- Is an important medical event when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Events **not** considered to be SAEs are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

11.1.3 Expectedness

AEs can be 'Expected' or 'Unexpected.'

- Expected AE

  Expected AEs are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an AE is considered expected when it appears in the current AE list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.

  Refer to Section 6.1 for a listing of expected AEs associated with the study agent(s).

- Unexpected AE

  For the purposes of this study, an AE is considered unexpected when it varies in nature, intensity or frequency from information provided in the current AE list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk.

11.1.4 Attribution

Attribution is the relationship between an AE or SAE and the study treatment. Attribution will be assigned as follows:

- Definite – The AE is clearly related to the study treatment.
- Probable – The AE is likely related to the study treatment.
- Possible – The AE may be related to the study treatment.
- Unlikely - The AE is doubtfully related to the study treatment.
- Unrelated - The AE is clearly NOT related to the study treatment.

11.2 Procedures for AE and SAE Recording and Reporting

Participating investigators will assess the occurrence of AEs and SAEs at all participant evaluation time points during the study.

All AEs and SAEs whether reported by the participant, discovered during questioning, directly observed, or detected by physical examination, laboratory test or other means, will be recorded in the participant's medical record and on the appropriate study-specific case report forms.

The descriptions and grading scales found in the revised NCI CTCAE version 4.0 will be utilized for AE reporting. 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 website at:
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

11.3 Reporting Requirements

The DF/HCC will serve as the coordinating center for this multi-site trial. Each participating investigator is required to abide by the reporting requirements set by the DF/HCC. The study must be conducted in compliance with FDA regulations, local safety reporting requirements, and reporting requirements of the principal investigator. Each investigative site will be responsible to report SAEs that occur at that institution to their respective IRB. It is the responsibility of each participating investigator to report SAEs to the study sponsor and/or others as described below.

11.4 Reporting to the Study Sponsor

11.4.1 SAE Reporting

All SAEs that occur after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment must be reported to the DF/HCC Overall PI on the local institutional SAE form. This includes events meeting the criteria outlined in Section 11.1.2, as well as the following:

- Grade 2 (moderate) and Grade 3 (severe) Events – Only events that are unexpected and possibly, probably or definitely related/associated with the intervention.

- All Grade 4 (life-threatening or disabling) Events – Unless expected AND specifically listed in the protocol as not requiring reporting.

- All Grade 5 (fatal) Events – When the participant is enrolled and actively participating in the trial OR when the event occurs within 30 days of the last study intervention.

Note: If the participant is in long term follow up, report the death at the time of continuing review.

Participating investigators must report each SAE to the DF/HCC Overall PI and Astellas Pharmaco-Vigilance Department within 24 hours of learning of the occurrence. In the event that the participating investigator does not become aware of the SAE immediately (e.g., participant sought treatment elsewhere), the participating investigator is to report the event within 24 hours after learning of it and document the time of his or her first awareness of the AE. Report SAEs by telephone, email or facsimile to:

[redacted]

Astellas Pharma Global Department – United States
[redacted]

Within the following 24-48 hours, the participating investigator must provide follow-up information on the SAE. Follow-up information should describe whether the event has resolved or continues, if and how the event was treated, and whether the participant will continue or discontinue study participation.

11.4.2 Non-SAE Reporting

Non-SAEs will be reported to the DF/HCC Overall PI on the toxicity CRFs.

11.5 Reporting to the IRBs

Investigative sites within DF/HCC will report all SAEs directly to the DFCI OHRS.

Other investigative sites should report SAEs to their respective IRB according to the local IRB's policies and procedures in reporting AEs. A copy of the submitted institutional SAE form should be forwarded to:

[redacted]

The DF/HCC PI will submit SAE reports from outside institutions to the DFCI OHRS according to DFCI IRB policies and procedures in reporting AEs.

11.6 Reporting to the FDA

This study is IND Exempt.

11.7 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any subject safety reports or sentinel events that require reporting according to institutional policy.

11.8 Monitoring of Adverse Events and Period of Observation

All AEs, both serious and non-serious, and deaths that are encountered from initiation of study intervention, throughout the study, and within 30 days of the last study intervention should be followed to their resolution, or until the participating investigator assesses them as stable, or the participating investigator determines the event to be irreversible, or the participant is lost to follow-up. The presence and resolution of AEs and SAEs (with dates) should be documented on the appropriate case report form and recorded in the participant's medical record to facilitate source data verification.

For some SAEs, the study sponsor or designee may follow-up by telephone, fax, and/or monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. Participating investigators should notify the DF/HCC Overall PI and their respective IRB of any unanticipated death or AE occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

12. DATA AND SAFETY MONITORING

12.1 Data Reporting

12.1.1 Method

The ODQ will collect, manage, and perform quality checks on the data for this study.

12.1.2 Data Submission

The schedule for completion and submission of case report forms (paper or electronic) to the ODQ is as follows:

Form	Submission Timeline
Eligibility Checklist	Complete prior to registration with ODQ.
On Study Form	Within 14 days of registration.
Baseline Assessment Form	Within 14 days of registration.
Treatment Form	Within 10 days of the last day of the cycle.
AE Report Form	Within 10 days of the last day of the cycle. If AEs are ongoing at the end of the last cycle, continue to submit AE reports until resolution or 30 days post-treatment.
Response Assessment Form	Within 10 days of the completion of the cycle required for response evaluation.
Off Treatment/Off Study Form	Within 14 days of completing treatment or being taken off study for any reason.
Follow up/Survival Form	Within 14 days of the protocol defined follow up visit date or call.

12.2 Safety Meetings

The DF/HCC DSMC will review and monitor toxicity and accrual data from this trial. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Principal Investigator and study team.

The DSMC will review each protocol up to four times a year or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; all grade 2 or higher unexpected AEs that have been reported; summary of all deaths occurring within 30 days for Phase I or II protocols; for gene transfer protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Monitoring

Involvement in this study as a participating investigator implies acceptance of potential audits or inspections, including source data verification, by representatives designated by the DF/HCC Overall PI (or Protocol Chair) or DF/HCC. The purpose of these audits or inspections is to examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy, GCP, and any applicable regulatory requirements.

All data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion.

See section 5.0 of the Data Safety Monitoring Plan (Appendix E) for additional details on monitoring of external sites for this study.

13. REGULATORY CONSIDERATIONS

13.1 Protocol Review and Amendments

This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB governing each study location.

Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The DF/HCC Overall PI (or Protocol Chair) will disseminate protocol amendment information to all participating investigators.

All decisions of the IRB concerning the conduct of the study must be made in writing.

13.2 Informed Consent

All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

13.3 Ethics and Research Practices

This study is to be conducted according to the following considerations, which represent good and sound research practice:

- US CFR governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki
    - Title 21 Part 50 – Protection of Human Subjects
    - [redacted]

- Title 21 Part 54 – Financial Disclosure by Clinical Investigators
- Title 21 Part 56 – Institutional Review Boards
- Title 21 Part 312 – Investigational New Drug Application

- State laws
- DF/HCC research policies and procedures

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB according to the local reporting policy.

13.4 Study Documentation

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified.

Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

13.5 Records Retention

All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

13.6 Multi-Center Guidelines

This protocol will adhere to the policies and requirements of the DF/HCC. The specific responsibilities of the DF/HCC Overall PI (or Protocol Chair), Coordinating Center, and Participating Institutions are presented in the Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (see Appendix E).

- The DF/HCC Overall PI/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports to all participating institutions for submission to their individual IRBs for action as required.

- Mechanisms will be in place to ensure quality assurance, protocol compliance, and AE reporting at each site.

- Except in very unusual circumstances, each participating institution will order the agent(s) directly from the supplier. A participating site may order the agent(s) only after the initial IRB approval for the site has been forwarded to the Coordinating Center.

14. STATISTICAL CONSIDERATIONS

14.1 Primary Analysis

The primary objective of this trial is to analyze possible AR related mechanisms of resistance to enzalutamide in serial CRPC biopsies including AR sequencing (mutations, splice variants), AR regulated gene expression, tumor androgen levels, profiling of enzymes involved in androgen synthesis/metabolism, and whole-exome and transcriptome sequencing. These data are of various forms resulting in different measures for a given participant: (a) continuous data will be evaluated by calculating mean percent change in level, (b) ordinal data will be evaluated by the mean percent change in count, and (c) categorical binary data will be evaluated by No/Yes change in status.

For the primary analysis, only participants who received at least 2 cycles of enzalutamide and experienced progression will be eligible. We plan to enroll a total of 66 participants to obtain 40 evaluable participants. A 60% overall yield is based on the assumptions that 95% of participants are eligible, 90% of these participants have paired biopsies and 70% of participants have evaluable tumors for each sample.

The target sample size is based on power to evaluate a mean percent change in level. The hypothesis is that AR reactivation can lead to resistance to enzalutamide. We will evaluate the mean percent change in a set of resistance parameters using the paired t-test. All continuous variables will be evaluated as a set. A mean percent change of 30% would be considered biologically significant. The effect size is defined as the mean percent change divided by the standard deviation (SD) of percent change, which is unknown in this population. The table below provides the mean percent change that can be detected with 80% or 90% power under various scenarios of SD given a one-sided 0.025 alpha and 40 evaluable participants. For example, there is 80% power to detect mean percent change of 22.7% given a SD of 50% corresponding to an effect size=0.454 SD. The mean percent change presented ranges from 13.6%-31.5%. Therefore, this study is designed with satisfactory power to show a biologically significant mean percent change in a set of resistance parameters.

Paired Tumor Samples (n=40)				
	Paired t-test, 1-sided alpha=0.025			
Power=80%, Effect Size=0.454 SD				
Standard Deviation	30%	40%	50%	60%
Mean Percent Change	13.6%	18.2%	22.7%	27.3%
Power=90%, Effect Size=0.526 SD				
Standard Deviation	30%	40%	50%	60%
Mean Percent Change	15.8%	21.0%	26.3%	31.5%

Number of Subjects/Study Duration:
We plan to enroll a total of 66 participants to obtain 40 evaluable participants. Assuming an accrual rate of 6 participants per month, enrollment will take 11 months. Participants will be treated with enzalutamide 160 mg orally per day. We will assess PSA at baseline and day 1 of each 28-day cycle until radiographic progression defined by PCWG2. Participants will continue on therapy until radiographic or symptomatic progression or taken off study for another reason.

14.2 Analysis of Secondary Endpoints

Safety and tolerability of enzalutamide will be evaluated. All participants who receive at least 1 dose of study drug will be included in these analyses. A summary of all Grade 1-4 treatment-related toxicities will be provided by toxicity type and maximum grade. The maximum grade consolidates the reports of a given type of toxicity for a participant over time by taking the maximum grade across time (i.e. a participant appears only once for a given type of toxicity). Participants with reports of multiple toxicities of different types are reported multiple times under the relevant toxicity categories. 'Treatment-related' toxicities are defined by an attribution as possible, probable or definite. With 66 participants, the maximum width of a 90% CI for a given toxicity rate is 22%. The probability of observing at least one rare severe toxicity (true rate=5%) is 95%. The number of cycles completed with and without dose modifications and treatment delays.

PSA response to enzalutamide will be summarized as frequency and percent. Participants who are unevaluable for response will be included in the denominator as nonresponders when calculating the response rate. Duration of PSA response for the regimen of enzalutamide will be defined relative to the first achievement of PSA response and will be estimated using the method of Kaplan and Meier. PSA response will be measured according to PCWG2 (2008) criteria.

Participants with measurable disease will be evaluated for response using RECIST 1.1 criteria. Duration of response of bone lesions or measurable disease will be described using Kaplan-Meier estimates**.**

The set of AR resistance measures will be summarized using descriptive statistics (mean, median, standard deviation (SD), interquartile range (IQR)). Correlation among measures will be assessed graphically with scatter plots and using Pearson correlation coefficients (or Spearman if appropriate).

The two-group t-test will be used to examine the difference in level and/or change pre- to post-treatment in levels by PSA and RECIST (measurable disease participants) response outcome. Assuming 40 evaluable participants, if PSA response rate is 25% (10 responders and 30 non-responders), there is 80% power to detect an effect of 1.05 SD at a two-sided significance level of 0.05. Continuous levels may be dichotomized at the median for further evaluation. Association between response and categorical variables will be evaluated using Fisher's exact test. Analyses of these correlative endpoints will be exploratory and adjustments will not be made for multiple comparisons.

15. PUBLICATION PLAN

The data will be collected by [redacted] and analyzed by [redacted] and the statistical team at DFCI. It is anticipated that the results will be made public within 12 months of the end of data collection. A report is planned to be published in a peer-reviewed journal, however initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors.

16. REFERENCES

1. Tran C, Ouk S, Clegg NJ, Chen Y, Watson PA, Arora V, et al. Development of a second-generation antiandrogen for treatment of advanced prostate cancer. Science. 2009;324(5928):787-90.
2. Joyce R, Fenton MA, Rode P, Constantine M, Gaynes L, Kolvenbag G, et al. High dose bicalutamide for androgen independent prostate cancer: effect of prior hormonal therapy. J Urol. 1998;159(1):149-53.
3. Kucuk O, Fisher E, Moinpour CM, Coleman D, Hussain MH, Sartor AO, et al. Phase II trial of bicalutamide in patients with advanced prostate cancer in whom conventional hormonal therapy failed: a Southwest Oncology Group study (SWOG 9235). Urology. 2001;58(1):53-8.
4. Scher HI, Liebertz C, Kelly WK, Mazumdar M, Brett C, Schwartz L, et al. Bicalutamide for advanced prostate cancer: the natural versus treated history of disease. J Clin Oncol. 1997;15(8):2928-38.
5. Kassouf W, Tanguay S, Aprikian AG. Nilutamide as second line hormone therapy for prostate cancer after androgen ablation fails. J Urol. 2003;169(5):1742-4.
6. Small EJ, Halabi S, Dawson NA, Stadler WM, Rini BI, Picus J, et al. Antiandrogen withdrawal alone or in combination with ketoconazole in androgen-independent prostate cancer patients: a phase III trial (CALGB 9583). J Clin Oncol. 2004;22(6):1025-33.
7. Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, et al. Molecular determinants of resistance to antiandrogen therapy. Nat Med. 2004;10(1):33-9.

8. Scher HI, Beer TM, Higano CS, Anand A, Taplin ME, Efstathiou E, et al. Antitumour activity of MDV3100 in castration-resistant prostate cancer: a phase 1-2 study. Lancet. 2010;375(9724):1437-46.

9. Scher HI, Fizazi K, Saad F, Taplin ME, Sternberg CN, Miller K, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med. 2012;367(13):1187-97.

10. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin. 2012;62(1):10-29.

11. Vasaitis TS, Bruno RD, Njar VC. CYP17 inhibitors for prostate cancer therapy. J Steroid Biochem Mol Biol. 2011;125(1-2):23-31.

12. Nakamura Y, Suzuki T, Fukuda T, Ito A, Endo M, Moriya T, et al. Steroid sulfatase and estrogen sulfotransferase in human prostate cancer. Prostate. 2006;66(9):1005-12.

13. Friedlander TW, Ryan CJ. Targeting the androgen receptor. Urol Clin North Am. 2012;39(4):453-64.

14. Attard G, Cooper CS, de Bono JS. Steroid hormone receptors in prostate cancer: a hard habit to break? Cancer Cell. 2009;16(6):458-62.

15. Reid AH, Attard G, Barrie E, de Bono JS. CYP17 inhibition as a hormonal strategy for prostate cancer. Nat Clin Pract Urol. 2008;5(11):610-20.

16. Small EJ, Halabi S, Dawson NA, Stadler WM, Rini BI, Picus J, et al. Antiandrogen withdrawal alone or in combination with ketoconazole in androgen-independent prostate cancer patients: a phase III trial (CALGB 9583). J Clin Oncol. 2004;22(6):1025-33.

17. 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(9):3550-5.

18. Visakorpi T, Hyytinen E, Koivisto P, Tanner M, Keinanen R, Palmberg C, et al. In vivo amplification of the androgen receptor gene and progression of human prostate cancer. Nat Genet. 1995;9(4):401-6.

19. Taplin ME, Bubley GJ, Shuster TD, Frantz ME, Spooner AE, Ogata GK, et al. Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer. N Engl J Med. 1995;332(21):1393-8.

20. Stanbrough M, Bubley GJ, Ross K, Golub TR, Rubin MA, Penning TM, et al. Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. Cancer Res. 2006;66(5):2815-25.

21. Guo Z, Yang X, Sun F, Jiang R, Linn DE, Chen H, 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(6):2305-13.

22. Hornberg E, Ylitalo EB, Crnalic S, Antti H, Stattin P, Widmark A, et al. Expression of androgen receptor splice variants in prostate cancer bone metastases is associated with castration-resistance and short survival. PLoS One. 2011;6(4):e19059.

23. Hu R, Dunn TA, Wei S, Isharwal S, Veltri RW, Humphreys E, et al. Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. Cancer Res. 2009;69(1):16-22.

24. Li Y, Alsagabi M, Fan D, Bova GS, Tewfik AH, Dehm SM. Intragenic rearrangement and altered RNA splicing of the androgen receptor in a cell-based model of prostate cancer progression. Cancer Res. 2011;71(6):2108-17.

25. Li Y, Chan SC, Brand LJ, Hwang TH, Silverstein KA, Dehm SM. Androgen receptor splice variants mediate enzalutamide resistance in castration-resistant prostate cancer cell lines. Cancer Res. 2013;73(2):483-9.

26. Scher HI, Halabi S, Tannock I, Morris M, Sternberg CN, Carducci MA, 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. J Clin Oncol. 2008;26(7):1148-59.

27. Stein MN, Goodin S, Dipaola RS. Abiraterone in prostate cancer: a new angle to an old problem. Clin Cancer Res. 2012;18(7):1848-54.

28. Mostaghel EA, Nelson PS. Intracrine androgen metabolism in prostate cancer progression: mechanisms of castration resistance and therapeutic implications. Best Pract Res Clin Endocrinol Metab. 2008;22(2):243-58.

29. Ryan CJ, Shah S, Efstathiou E, Smith MR, Taplin ME, Bubley GJ, et al. Phase II study of abiraterone acetate in chemotherapy-naive metastatic castration-resistant prostate cancer displaying bone flare discordant with serologic response. Clin Cancer Res. 2011;17(14):4854-61.

17. APPENDICES

Appendix A: Performance Status Criteria

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

Appendix B: Required Forms at Registration

The DFCI coordinator will Fax to DFCI ODQ:

- Current IRB approved consent form signed by participant and Investigator (MD only)
- HIPAA authorization form (if separate from the informed consent document)
- Signed and dated DFCI eligibility checklist (signed by MD or RN)
- The following source documentation is typically required:
- Please note: Additional documentation may be required by the lead institution.
    - Labs for PSA values used to determine eligibility (Lab values used to determine eligibility, including screening PSA)
    - Documentation of prior treatments/procedures performed to treat prostate cancer (e.g. Chemotherapy, Cryotherapy, Hormone Therapy,
    - Radiation therapy with start and stop dates and dosing information if applicable)
    - Reports documenting disease status
        - Chest CT
        - CT or MRI Abdomen and Pelvis
        - Bone Scan
    - Pathology Report
    - Concomitant medication list
    - Progress note or equivalent documentation of consenting visit
    - Progress note documenting medical history and oncologic history
    - Screening Labs
        - Complete Blood Count with differential
        - Electrolytes
        - Liver Function Tests
        - Total Testosterone
        - PSA
        - Coagulation Factors
    - Screening visit note, with BP, vital signs, ECOG Performance status
    - Screening ECG

Appendix C: Participant's Pill Diary

Today's Date: _____ Participant Study ID: _____
Participant Name: _____ Cycle Number: _____

INSTRUCTIONS TO THE PARTICIPANT:

1. Complete one form for each cycle (28 days).
2. You will take ____ capsules each day by mouth.
3. Record the date, the number of capsules you took, and when you took them.
4. If you have any comments or notice any side effects, please record them in the comments column.
5. Please bring your pill bottle and this form to your physician when you go to your next appointment.

Date	Day	Number of Capsules	Time	Comments	Date	Day	Number of Capsules	Time	Comments
	1					15			
	2					16			
	3					17			
	4					18			
	5					19			
	6					20			
	7					21			
	8					22			
	9					23			
	10					24			
	11					25			
	12					26			
	13					27			
	14					28			

Participant's Signature: _____ Date: _____

Physician's office will complete this section:
Date participant started protocol treatment:
_____
Date participant was removed from study:
_____
Participant's planned daily dose:
_____
Total number of pills taken this month: _____
Physician/Nurse/Data Manager's Signature: _____ Date: _____

Appendix D: Representative Medications that May Predispose to Seizure

Classification	Generic Name	Trade Name
Antiasthmatic drugs	Aminophylline	Neophyllin, Albina, Kyophyllin, etc
	Theophylline	Theodur, Uniphyl, etc.
Atypical antipsychotics	Clozapine	Clozaril, etc.
	Olanzapine	Zyprexa, etc.
	Risperidone	Risperdal, etc.
	Quetiapine	Seroquel, etc.
	Perospirone	Lullan, etc.
	Aripiprazole	Abilify, etc.
	Blonanserin	Lonasen, etc.
Insulin	Insulin aspart	Novorapid, etc.
	Insulin lispro	Humalog, etc.
	Insulin glulisine	Apidra, etc.
	Human insulin	Humulin, Novolin, etc.
	Insulin glargine	Lantus, etc.
	Insulin detemir	Levemir, etc.
Lithium	Lithium carbonate	Limas, etc.
Pethidines	Pethidine hydrochloride	Pethidine hydrochloride, Opystan, Pethilorfan, etc.
Phenothiazine antipsychotics	Chlorpromazine	Wintermin, Contomin, Vegetamin, etc.
	Trifluoperazine	Trifluoperazine powder, etc.
	Levomepromazine	Hirnamin, Levotomin, etc.
	Fluphenazine	Flumezin, Fludecasin, etc.
	Prochlorperazine	Novamin, Compazine, etc.
	Propericiazine	Apamin, Neuleptil, etc.
	Perphenazine	PZC, Trilafon, etc.
Certain antidepressants	Bupropion	Wellbutrin, Xyban, etc.
	Amitriptyline	Tryptanol, etc.
	Imipramine	Imidol, Tofranil, etc.
	Maprotiline	Ludiomil, etc.
	Mirtazapine	Reflex, Remeron, etc.
	Amoxapine	Amoxan, etc.
	Dosulepin hydrochloride	Prothiaden, etc.
	Nortriptyline hydrochloride	Noritren, etc.
	Lofepramine hydrochloride	Amplit, etc.
	Mianserin hydrochloride	Tetramide, etc.
	Setiptiline maleate	Tecipul, etc.
	Trimipramine maleate	Surmontil, etc.
	Clomipramine hydrochloride	Anafranil, etc.

APPENDIX E
Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan

TABLE OF CONTENTS

1.0 INTRODUCTION

The Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (DF/HCC DSMP) outlines the procedures for conducting a DF/HCC Multi-Center research protocol. The DF/HCC DSMP should serve as a reference for any sites external to DF/HCC that will be participating in the research protocol.

1.1 Purpose

To establish standards that will ensure that a Dana-Farber/Harvard Cancer Center Multi-Center protocol will comply with Federal Regulations, Health Insurance Portability and Accountability Act (HIPAA) requirements and applicable DF/HCC Standard Operating Procedures

1.2 Multi-Center Data and Safety Monitoring Plan Definitions

DF/HCC Multi-center Protocol: A research protocol in which one or more outside institutions are collaborating with Dana-Farber/Harvard Cancer Center where a DF/HCC investigator is the sponsor. DF/HCC includes Dana-Farber/Partners Cancer Care (DF/PCC) Network Clinical Trial Affiliates.

Lead Institution: One of the Dana-Farber/Harvard Cancer Center consortium members (Dana-Farber Cancer Institute (DFCI), Massachusetts General Hospital (MGH), Beth Israel Deaconess Medical Center (BIDMC), Children's Hospital Boston (CHB), Brigham and Women's Hospital (BWH)) responsible for the coordination, development, submission, and approval of a protocol as well as its subsequent amendments per the DFCI IRB and applicable regulatory guidelines (Food and Drug Administration (FDA,). The Lead Institution is typically the home of the DF/HCC Sponsor. The Lead Institution also typically serves as the Coordinating Center for the DF/HCC Multi-Center Protocol.

DF/HCC Sponsor: The person sponsoring the submitted Multi-Center protocol. Within DF/HCC, this person is the Overall Principal Investigator who takes responsibility for initiation, management and conduct of the protocol at all research locations. In applicable protocols, the DF/HCC Sponsor will serve as the single liaison with any regulatory agencies (i.e. FDA). The DF/HCC Sponsor has ultimate authority over the protocol and is responsible for the conduct of the study at DF/HCC and all Participating Institutions. In most cases the DF/HCC Sponsor is the same person as the DF/HCC Principal Investigator; however, both roles can be filled by two different people.

Participating Institution: An institution that is outside the DF/HCC and DF/PCC consortium that is collaborating with DF/HCC on a protocol where the sponsor is a DF/HCC Investigator. The Participating Institution acknowledges the DF/HCC Sponsor as having the ultimate authority and responsibility for the overall conduct of the study.

Coordinating Center: The entity (i.e. Lead Institution) that provides administrative

support to the DF/HCC Sponsor in order that he/she may fulfill the responsibilities outlined in the protocol document and DSMP, and as specified in applicable regulatory guidelines. In general, the Lead Institution is the Coordinating Center for the DF/HCC Multi-Center Protocol. Should the DF/HCC Sponsor decide to use a CRO, the CRO will be deemed the Coordinating Center.

DF/HCC Office of Data Quality: A unit within DF/HCC developed to computerize and manage data, and to provide a Quality Control and Quality Assurance function for DF/HCC trials.

2.0 GENERAL ROLES AND RESPONSIBILITIES

For DF/HCC Multi-Center Protocols, the DF/HCC Sponsor, the Coordinating Center, and the Participating Institutions are expected to adhere to the following general responsibilities:

2.1 DF/HCC Sponsor

The DF/HCC Sponsor, [redacted] will accept responsibility for all aspects of conducting a DF/HCC Multi-Center protocol which includes but is not limited to:

- Oversee the coordination, development, submission, and approval of the protocol as well as subsequent amendments.
- Ensure that the investigators, study team members, and Participating Institutions are qualified and appropriately resourced to conduct the protocol.
- Submit the Multi-Center Data and Safety Monitoring Plan as an appendix to the protocol.
- Assure all Participating Institutions are using the correct version of the protocol.
- Ensure that each participating investigator and study team receives adequate protocol training and/or a Site Initiation Visit prior to enrolling participants and throughout trial's conduct as needed.
- Ensure the protocol will be provided to each participating site in a language understandable to all site personnel when English is not the primary language.
- Monitor progress and overall conduct of the study at all Participating Institutions.
- Ensure all DFCI Institutional Review Board (IRB), DF/HCC and other applicable (FDA) reporting requirements are met.
- Review data and maintain timely submission of data for study analysis.
- Act as the single liaison with FDA (investigator-held IND trials) as applicable.
- Ensure compliance with all requirements as set forth in the Code of Federal Regulations, applicable DF/HCC requirements, HIPAA requirements, and the approved protocol.
- Commit to the provision that the protocol will not be rewritten or modified by anyone other than the DF/HCC Sponsor.
- Identify and qualify Participating Institutions and obtain accrual commitments prior to extending the protocol to that site.

2.2 Coordinating Center

The Coordinating Center will assume the following general responsibilities:

- Assist in protocol development
- Maintain copies of Federal Wide Assurance and Institutional Review Board (IRB) approvals from all Participating Institutions.
- Maintain FDA correspondence, as applicable.
- Maintain updated roster of participants.
- Verify eligibility.
- Verify response.
- Oversee the data collection process from Participating Institutions.
- Maintain documentation of Serious Adverse Event (SAE) reports submitted by Participating Institutions and submit to DF/HCC Sponsor for timely review.
- Distribute adverse events reported to the DF/HCC Sponsor that fall under the DFCI IRB Adverse Event Reporting Policy to all participating investigators.
- Provide Participating Institutions with information regarding DF/HCC requirements that they will be expected to comply with.
- Monitor Participating Institutions either by on-site or virtual monitoring.
- Maintain Regulatory documents of all Participating Institutions.
- Conduct regular communications with all Participating Institutions (conference calls, emails, etc).
- Maintain documentation of all communications.
- Ensure that each Participating Institution has the appropriate assurance on file with the Office of Human Research Protection (OHRP).

2.3 DF/HCC Office of Data Quality (ODQ)

In addition to the Coordinating Center, the DF/HCC ODQ provides the following support services to assist the DF/HCC Sponsor:

- Develop protocol specific case report forms (CRF/eCRFS).
- QA/QC data of protocol specific CRFs.
- Provide a central participant registration, which includes review of consent and eligibility.
- Provide auditing services (funding and ODQ approval required).

2.4 Participating Institution

Each Participating Institution is expected to comply with all applicable Federal Regulations and DF/HCC requirements, the protocol and HIPAA requirements. All Participating Institutions will provide a list of personnel assigned to the role for oversight of data management at their site to the Coordinating Center.

The general responsibilities for each Participating Institution are as follows:

- Commit to the accrual of participants to the protocol.

- Submit protocol and/or amendments to their local IRB.
- Maintain a regulatory binder in accordance with DF/HCC requirements.
- Provide the Coordinating Center with regulatory documents as requested.
- Participate in protocol training prior to enrolling participants and throughout the trial as needed (i.e. teleconferences).
- Update Coordinating Center with research staff changes on a timely basis.
- Register participants through the Coordinating Center.
- Submit source documents, research records, and CRFs per protocol specific submission guidelines to the Coordinating Center.
- Submit Serious Adverse Event (SAE) reports to local IRB per local requirements and to the Coordinating Center, in accordance with DF/HCC requirements.
- Submit protocol deviations and violations to local IRB per local requirements and to the DF/HCC Sponsor in accordance with DF/HCC requirements.
- Secure and store investigational agents and/or other protocol mandated drugs per federal guidelines and protocol requirements.
- Have office space, office equipment, and internet access that meet HIPAA standards.

3.0 DF/HCC REQUIREMENTS FOR MULTI-CENTER PROTOCOLS

The following section will clarify DF/HCC Requirements and further detail the expectations for participating in a DF/HCC Multi-Center protocol.

3.1 Protocol Distribution

The Coordinating Center will distribute the final DFCI IRB approved protocol and any subsequent amended protocols to all Participating Institutions.

3.2 Protocol Revisions and Closures

The Participating Institutions will receive notification of protocol revisions and closures from the Coordinating Center. It is the individual Participating Institution's responsibility to notify its IRB of these revisions.

- **Non life-threatening revisions:** Participating Institutions will receive written notification of protocol revisions regarding non life-threatening events from the Coordinating Center. Non-life-threatening protocol revisions must be IRB approved and implemented within 90 days from receipt of the notification.

- **Revisions for life-threatening causes:** Participating Institutions will receive immediate notification from the Coordinating Center concerning protocol revisions required to protect lives with follow-up by fax, mail, e-mail, etc. Life-threatening protocol revisions will be implemented immediately followed by IRB request for approval.

- **Protocol closures and temporary holds:** Participating Institutions will receive notification of protocol closures and temporary holds from the Coordinating Center. Closures and holds will be effective immediately. In addition, the Coordinating Center, will update the Participating Institutions on an ongoing basis about protocol accrual data so that they will be aware of imminent protocol closures.

3.3 Informed Consent Requirements

The DF/HCC approved informed consent document will serve as a template for the informed consent for Participating Institutions. The Participating Institution consent form must follow the consent template as closely as possible and should adhere to specifications outlined in the DF/HCC Guidance Document on Model Consent Language for PI-Initiated Multi-Center Protocols. This document will be provided separately to each Participating Institution.

Participating Institutions are to send their version of the informed consent document and HIPAA authorization, if a separate document, to the Coordinating Center for review and approval prior to submission to their local IRB. The approved consent form must also be submitted to the Coordinating Center after approval by the local IRB.

The Principal Investigator (PI) at each Participating Institution will identify the physician members of the study team who will be obtaining consent and signing the consent form for therapeutic protocols. Participating institutions must follow the DF/HCC requirement that only attending physicians obtain informed consent and re-consent to interventional trials (i.e. drug and/or device trials).

3.4 IRB Documentation

The following must be on file with the Coordinating Center:

- Approval letter of the Participating Institution's IRB
- Copy of the Informed Consent Form approved by the Participating Institution’s IRB
- Participating IRB’s approval for all amendments

It is the Participating Institution's responsibility to notify its IRB of protocol amendments. Participating Institutions will have 90 days from receipt to provide the Coordinating Center their IRB approval for amendments to a protocol.

3.5 IRB Re-Approval

Verification of IRB re-approval from the Participating Institutions is required in order to continue research activities. There is no grace period for continuing approvals.

The Coordinating Center will not register participants if a re-approval letter is not received from the Participating Institution on or before the anniversary of the previous approval date.

3.6 Participant Confidentiality and Authorization Statement

In 1996, congress passed the first federal law covering the privacy of health information known as the Health Insurance Portability and Accountability Act (HIPAA). Any information, related to the physical or mental health of an individual is called Protected Health Information (PHI). HIPAA outlines how and under what circumstances PHI can be used or disclosed.

In order for covered entities to use or disclose protected health information during the course of a study, the study participant must sign an Authorization. This Authorization may or may not be separate from the informed consent document. The Coordinating Center, with the approval from the DFCI IRB, will provide a consent template, which covered entities (Participating Institutions) must use.

The DF/HCC Sponsor will use all efforts to limit its use of protected health information in its trials. However, because of the nature of these trials, certain protected health information must be collected per NCI requirements. These are the primary reasons why DF/HCC has chosen to use Authorizations, signed by the participant in the trial, rather than limited data sets with data use agreements.

3.6.1 DF/HCC Multi-Center Protocol Confidentiality

All documents, investigative reports, or information relating to the participant are strictly confidential. Whenever reasonably feasible, any participant specific reports (i.e. Pathology Reports, MRI Reports, Operative Reports, etc.) submitted to the Coordinating Center must have the participant's full name & social security number "blacked out" and the assigned DF/HCC ODQ case number (as described below) and DF/HCC protocol number written in (with the exception of the signed informed consent document). Participant initials may only be included or retained for cross verification of identification

3.7 DF/HCC Multi-Center Registration

3.7.1 Participant Registration and Randomization

To register a participant, the following documents should be completed by the Participating Institution and faxed to the Coordinating Center at Dana-Farber Cancer Institute at [redacted] or e-mailed to the DFCI Clinical Research Coordinator and Research Nurse team.

- Current IRB approved informed consent document signed by participant and

investigator
- HIPAA authorization form (if separate from the informed consent document)
- Signed and dated DFCI eligibility checklist
- The following source documentation is typically required. Please note additional documentation may be required by the lead institution:
  - Labs for PSA values used to determine eligibility (lab values used to determine eligibility, including screening PSA)
  - Documentation of prior treatments/procedures performed to treat prostate cancer (e.g. Chemotherapy, Cryotherapy, Hormone Therapy, Radiation therapy with start and stop dates and dosing information if applicable)
  - Reports documenting disease status*:* Chest CT**,** CT or MRI Abdomen and Pelvis**.** Bone Scan
  - Pathology Report
  - Concomitant medication list
  - Progress note or equivalent documentation of consenting visit
  - Progress note documenting medical history and oncologic history
  - All screening labs
  - Screening visit note, with BP, vital signs, ECOG Performance status
  - Screening ECG

The Coordinating Center will review the submitted documents in order to verify eligibility and consent. To complete the registration process, the Coordinating Center will:
- *Register the participant on the study with the DF/HCC Clinical Trial Management System (CTMS).*
- Upon receiving confirmation of registration the Coordinating Center will inform the Participating Institution and provide the study specific participant case number, and if applicable the dose treatment level.

Treatment may not begin without confirmation from the Coordinating Center that the participant has been registered.

Randomization can only occur during normal business hours, Monday through Friday from 8:00 AM to 5:00 PM Eastern Time.

3.7.2 Initiation of Therapy

Participants must be registered with the DF/HCC CTMS before receiving treatment. Treatment may not be initiated until the Participating Institution receives a faxed or e-mailed copy of the participant's registration confirmation memo from the Coordinating Center. Therapy must be initiated per protocol guidelines. The DF/HCC Sponsor and DFCI IRB must be notified of any exceptions to this policy.

3.7.3 Eligibility Exceptions

No exceptions to the eligibility requirements for a protocol without DFCI IRB approval will be permitted. All Participating Institutions are required to fully comply with this requirement. The process for requesting an eligibility exception is defined below.

3.7.4 Verification of Registration, Dose Levels, and Arm Designation

A registration confirmation memo for participants registered to DF/HCC Multi-Center Protocol will be faxed or emailed to the registering institution within one business day of the registration. Treatment may not be initiated until the site receives a faxed or e-mailed copy of the registration confirmation memo.

3.8 DF/HCC Protocol Case Number

At the time of registration, the following identifiers are required for all subjects: initials, date of birth, gender, race and ethnicity. Once eligibility has been established and the participant successfully registered, the participant is assigned a unique protocol case number. Participating Institutions should submit all de-identified subsequent communication and documents to the Coordinating Center, using this case number to identify the subject.

3.9 Protocol Deviations, Exceptions and Violations

Federal Regulations require an IRB to review proposed changes in a research activity to ensure that researchers do not initiate changes in approved research without IRB review and approval, except when necessary to eliminate apparent immediate hazards to the participant. DF/HCC requires all departures from the defined procedures set forth in the IRB approved protocol to be reported to the DF/HCC Sponsor, who in turn is responsible for reporting to the DFCI IRB.

For reporting purposes, DF/HCC uses the terms “violation”, “deviation” and “exception” to describe derivations from a protocol. All Participating Institutions must adhere to these requirements for reporting to the DF/HCC Sponsor and will follow their institutional policy for reporting to their local IRB.

3.9.1 Definitions

Protocol Deviation: Any departure from the defined procedures set forth in the IRB-approved protocol which is *prospectively approved* prior to its implementation.

Protocol Exception: Any protocol deviation that relates to the eligibility criteria, e.g. enrollment of a participant who does not meet all inclusion/exclusion criteria.

Protocol Violation: Any protocol deviation that was not *prospectively approved* by the IRB prior to its initiation or implementation.

3.9.2 Reporting Procedures

DF/HCC Sponsor: is responsible for ensuring that clear documentation is available in the medical record and/or regulatory documents to describe all protocol exceptions, deviations and violations. The DF/HCC Sponsor will also be responsible for ensuring that all protocol violations/deviations are promptly reported per DFCI IRB guidelines.

Participating Institutions: Protocol deviations require prospective approval from the DFCI IRB. The Participating Institution must submit the deviation request to the Coordinating Center who will then submit the deviation request to the DFCI IRB. Upon DFCI IRB approval the deviation is submitted to the Participating Institution IRB, per institutional policy. A copy of the Participating Institution's IRB report and determination will be forwarded to the Coordinating Center within 10 business days after the original submission.

All protocol violations must be sent to the Coordinating Center in a timely manner.

Coordinating Center: Upon receipt of the violation/deviation report from the Participating Institution, the Coordinating Center will submit the report to the DF/HCC Sponsor for review. Subsequently, the Participating Institution's IRB violation/deviation report will be submitted to the DFCI IRB for review per DFCI IRB reporting guidelines.

3.10 Safety Assessments and Toxicity Monitoring

The study teams at all participating institutions are responsible for protecting the safety, rights and well-being of study participants. Recording and reporting of adverse events that occur during the course of a study help ensure the continuing safety of study participants.

All participants receiving investigational agents and/or other protocol mandated treatment will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical examination findings, and spontaneous reports of adverse events reported by participants. All toxicities encountered during the study will be evaluated according to the NCI criteria specified in the protocol. Life-threatening toxicities must be reported immediately to the DF/HCC Sponsor via the Coordinating Center.

Additional safety assessments and toxicity monitoring will be outlined in the protocol.

3.10.1 Guidelines for Reporting Serious Adverse Events

Guidelines for reporting Adverse Events (AEs) and Serious Adverse Events (SAEs) are detailed in protocol section 11.

Participating Institutions must report the AEs to the DF/HCC Sponsor and the Coordinating Center following the DFCI IRB SAE Reporting Requirements.

The Coordinating Center will maintain documentation of all Participating Institution Adverse Event reports and be responsible for communicating to all participating

investigators, any observations reportable under the DFCI IRB Reporting Requirements. Participating Investigators will review any distributed AE reports, send a copy to their IRB according to their local IRB's policies and procedures, and file a copy with their regulatory documents.

3.10.2 Guidelines for Processing IND Safety Reports

FDA regulations require sponsors of clinical studies to notify the FDA and all participating investigators of any adverse experience associated with the use of the investigational agent that is both serious and unexpected. The DF/HCC Sponsor will review all IND Safety Reports and ensure that all IND Safety Reports are distributed to the Participating Institutions. The Participating Investigators are to review, send a copy to their IRB according to their local IRB's policies and procedures, and file a copy with their regulatory documents.

3.11 Data Management

The DF/HCC ODQ develops a set of either paper or electronic case report forms (CRF/eCRFs), for use with the protocol. These forms are designed to collect data for each study. The DF/HCC ODQ provides a web based training for eCRF users. See section 12 of protocol.

3.11.1 Data Forms Review

When data forms arrive at the DF/HCC ODQ, they are reviewed for completeness, protocol treatment compliance, adverse events (toxicities) and response. Data submissions are monitored for timeliness and completeness of submission. Participating Institutions are notified of their data submission delinquencies in accordance with the following:

Incomplete or Questionable Data

If study forms are received with missing or questionable data, the submitting institution will receive a written or electronic query from the DF/HCC ODQ Data Analyst or study monitor. Responses to all queries should be completed and submitted within 14 calendar days. Responses may be returned on the written query or on an amended paper case report form, or in the case of electronic queries, within the electronic data capture (eDC) system. In the case of a written query for data submitted on a paper case report form, the query must be attached to the specific data being re-submitted in response.

Missing Forms

If study forms are not submitted on schedule, the Participating Institution will receive a Missing Form Report from the Coordinating Center noting the missing forms. These reports are compiled by the DF/HCC ODQ and distributed a minimum of four times a year.

4.0 REQUSITIONING INVESTIGATIONAL DRUG

The ordering of investigational agent is specified in the protocol section 7.19.

Participating Institutions should order their own agent regardless of the supplier (i.e. pharmaceutical company.)

5.0 MONITORING: QUALITY CONTROL

The quality control process for a clinical trial requires verification of protocol compliance and data accuracy. The Coordinating Center, with the aid of the ODQ provides quality control oversight for the protocol.

5.1 Ongoing Monitoring of Protocol Compliance

The Participating Institutions will be required to submit participant source documents to the Coordinating Center for monitoring upon request. Participating Institution may also be subject to on-site monitoring conducted by the Coordinating Center.

Source documents confirming eligibility are to be sent to DFCI by the participating institutions and reviewed by DFCI study staff including a clinician prior to external site participant registration.

The DF/HCC Lead Institution will implement monitoring activities ongoing to ensure that Participating Institutions are complying with regulatory and protocol requirements, data quality, and subject safety. Additional monitoring practices may include but are not limited to; source verification, review and analysis of the following: eligibility requirements of all participants , informed consent procedures, adverse events and all associated documentation, study drug administration / treatment, regulatory records and site trial master files, protocol deviations, pharmacy records, response assessments, and data management.

Site visits will generally occur once a year for sites that are actively enrolling participants and have participants in treatment. Virtual monitoring (source documents are sent to DFCI for review) may be performed in lieu of a site visit if the study staff and PI determine that virtual monitoring is appropriate for the site. The decision to perform virtual monitoring in lieu of a site visit will be based upon the site's enrollment, study compliance history, history collaborating with DFCI on other multi-center studies, and number of participants in active treatment.

We hope to monitor approximately 50% of participants enrolled from external sites. The target number of participants monitored may change depending upon monitor findings.

Monitoring will occur before the clinical phase of the protocol begins and will continue during protocol performance through study completion.

All data submitted to the DF/HCC ODQ will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. The Lead Institution or designee and if applicable ODQ Data Analysts assigned to the Protocol will perform the ongoing protocol data compliance monitoring with the support of the Participating Institution's Coordinators, the Principal Investigators, and the Protocol Chair.

Teleconferences between DFCI and the participating sites will be conducted on approximately a monthly basis. Meeting minutes for teleconferences will be issued to all participating sites. Site initiation visits will be conducted via teleconference. Ongoing training will also be conducted via teleconference as needed. The Coordinating Center, Dana Farber Cancer Institute will be available to all participating sites for resolving questions, concerns and facilitating compliance.

5.2 Evaluation of Participating Institution Performance

5.2.1 Monitoring Reports

The DF/HCC Sponsor will review all monitoring reports for on-site and virtual monitoring of Participating Institutions to ensure protocol compliance and ability to fulfill responsibilities of participating in the study. The DF/HCC Sponsor may increase the monitoring activities at Participating Institutions that are unable to comply with the protocol, DF/HCC Sponsor requirements or federal and local regulations. Participating Institutions may also be subject to an audit as determined by the DF/HCC Sponsor.

6.0 AUDITING: QUALITY ASSURANCE

Auditing is a method of Quality Assurance. Its main focus is to measure whether standards and procedures were followed. Auditing is the systematic and independent examination of all trial related activities and documents. Audits determine if evaluated activities were appropriately conducted and whether data was generated, recorded and analyzed, and accurately reported per the protocol, Standard Operating Procedures (SOPs), and the Code of Federal Regulations (CFR).

6.1 DF/HCC Sponsored Trials

An audit may be performed by the ODQ at the request of the DF/HCC Sponsor if serious instances of non-compliance are found during routine monitoring.

6.2 Participating Institution

It is the Participating Institution's responsibility to notify the Coordinating Center of all scheduled audit dates (internal or NCI) and re-audit dates (if applicable), which involve this protocol. All institutions will forward a copy of final audit and/or re-audit reports and corrective action plans (if applicable) to the Coordinating Center, within 12 weeks after the audit date.

6.3 DF/HCC Sponsor and Coordinating Center

The DF/HCC Sponsor will review all final audit reports and corrective action plans if applicable. The Coordinating Center, must forward these reports to the DF/HCC ODQ per DF/HCC policy for review by the DF/HCC Audit Committee. Based upon the audit assessments the DF/HCC Audit Committee could accept or conditionally accept the audit rating and final report. Conditional approval could require the DF/HCC Sponsor to implement recommendations or require further follow-up. For unacceptable audits, the DF/HCC Audit Committee would forward the final audit report and corrective action plan to the DFCI IRB as applicable.

6.4 Sub-Standard Performance

The DF/HCC Sponsor, DFCI IRB, is charged with considering the totality of an institution's performance in considering institutional participation in the protocol.

6.4.1 Corrective Actions

Participating Institutions that fail to meet the performance goals of accrual, submission of timely accurate data, adherence to protocol requirements, and compliance with state and federal regulations, will be recommended for a six-month probation period. Such institutions must respond with a corrective action plan and must demonstrate during the probation period that deficiencies have been corrected, as evidenced by the improved performance measures. Participating Institutions that fail to demonstrate significant improvement will be considered by the DF/HCC Sponsor for revocation of participation.