Abbreviated Title: Neoadjuvant ADT & Enzalutamide
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Title: Neoadjuvant Androgen Deprivation and Enzalutamide: Using Multiparametric MRI to Evaluate Intraprostatic Tumor Responses and Androgen Resistance Patterns in Newly Diagnosed Prostate Cancer

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Commercial Agents: Goserelin, Enzalutamide

PRÉCIS

Background:

- Most men diagnosed with prostate cancer will present with intermediate or high-risk disease.
- Many develop castrate resistant prostate cancer (CRPC) as curative strategies are often unsuccessful.
- Treatment options typically involve radical prostatectomy (RP) or radiation therapy (RT) in combination with androgen deprivation therapy (ADT).
- Even when cancers are initially sensitive to ADT, resistance ultimately emerges either through clonal selection or through a variety of adaptive mechanisms (secondary resistance).
- The recent introduction of novel androgen pathway inhibitors offers an opportunity to potentially improve the cure rate of men with intermediate and high risk localized prostate cancer.
- There remains a great need for improved techniques to determine mechanisms of treatment response and resistance.

Objectives:

- To test the feasibility of multi parametric magnetic resonance imaging (mpMRI) for the localization and detection of focal prostate cancer both before and after pre-operative treatment with ADT and enzalutamide.

Eligibility:

- Patients with nonmetastatic castration sensitive prostate cancer with intermediate or high-risk features
- Patients with testosterone levels ≥ 100 ng/dL
- ECOG 0-1

Design:

- Patients will be treated with ADT and enzalutamide for 6 months
- Two 3T mpMRI endorectal examinations (One at screening and after 6 month of treatment)
- Screening biopsy (MR/US guided) samples
- Standard of care prostatectomy (RP) following post treatment mpMRI
- All tumor specimens will undergo genomic analysis

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- To test the feasibility of mpMRI for the localization and detection of focal prostate cancer both before and after pre-operative treatment with ADT and enzalutamide.

1.1.2 Secondary Objectives

- To perform parallel sequencing of each unique tumor focus in the prostate before and after treatment with ADT and enzalutamide to determine the genetic predictors of primary resistance. (This will be done both inter-patient and intra-patient.)
- To determine the quantitative and qualitative mpMRI characteristics of response both before and after treatment, to predict resistance to intensive androgen targeted therapies and link imaging to genomic characterization of resistance.
- To evaluate the pathological complete response rate after neoadjuvant treatment with ADT and enzalutamide.

1.2 BACKGROUND AND RATIONALE

It is expected that 29, 480 men will die from prostate cancer in the US in 2014.[1] The majority of these men will present with intermediate or high risk localized cancer. While some of these men are cured, many men (20 – 40% of those diagnosed) will ultimately develop castrate resistant prostate cancer (CRPC) and succumb to the disease.[2] Treatment options typically involve radical prostatectomy (RP) or radiation therapy (RT) in combination with androgen deprivation therapy (ADT). Following RP upwards of 50% of patients with high-risk disease will experience a biochemical recurrence at 5 years,[3] and approximately 20% will die of their disease in 10-15 years.[4]

ADT interferes with androgen production or blocks androgen at the level of the androgen receptor (AR).[5] As a consequence prostate cancer growth is suppressed and apoptosis occurs. There is great variability in prostate cancers' sensitivity to ADT and the mechanism of induction of apoptosis when it occurs is poorly understood. Some tumors appear to exhibit primary resistance, meaning the tumor persists despite ADT. Even when cancers are initially sensitive to ADT, resistance ultimately emerges either through clonal selection or through a variety of adaptive mechanisms (secondary resistance).[6] Thus, the duration of long-term response to ADT is unpredictable. Due to the lack of *in vivo* markers of tumor response it has not been possible to predict either the inherent cellular properties of resistance (primary resistance) or the timing of conversion to castration resistance prostate cancer (secondary resistance).

The recent introduction of novel androgen pathway inhibitors offers an opportunity to potentially improve the cure rate of men with intermediate and high risk localized prostate cancer but has also creates a need for improved techniques to determine mechanisms of treatment response and resistance. It is this challenge that we seek to address in this clinical trial by linking pathologic tumor response to both genomics and imaging to predict androgen resistance. We will examine the response of tumors to goserelin and enzalutamide in the preoperative setting.

1.2.1 Preliminary experience with ADT prior to prostatectomy

In a previous study, the 17 α-hydroxylase/C17,20 lyase inhibitor abiraterone +ADT prior to prostatectomy yielded rapid PSA declines (including near or complete responses), however approximately two-thirds of studied patients demonstrated little or no tumor shrinkage.[7] This suggests that primary resistance to even modern anti-androgen therapy remains a formidable challenge. Several mechanisms of resistance to ADT have been identified, including persistent ligand and androgen receptor mutation.[6, 8] Despite this, the genomic determinants of resistance to ADT remain incompletely characterized. The extent to which such mechanisms might generalize across ADT regimens (such as ADT + Enzalutamide) or operate in specific therapeutic contexts also remains unknown.

1.2.2 Rationale for Enzalutamide in the Neoadjuvant Setting

The clinical importance of enzalutamide in the treatment of prostate cancer is likely just emerging. Given its ability to improve survival (in both chemotherapy-naïve patients and patients who have already received docetaxel), its relative lack of toxicity, and no requisite prednisone, there is increasing interest to use this treatment earlier in the disease process. One possible therapeutic strategy could employ enzalutamide in the neoadjuvant setting. It would be interesting to determine if enzalutamide can induce a complete pathologic response in some patients, or significantly shrink tumors (identified via endo-rectal MRI) in other patients. Associations with these pathologic and/or radiographic changes and delayed recurrence or cure could drive additional studies in the future with clinical endpoints.

1.2.3 Possible Mechanisms of Resistance in Prostate Cancer

There are multiple mechanisms of resistance that have been proposed in prostate cancer, but it remains unclear which are most prevalent and relevant in newly diagnosed patients. In treatment naïve patients it is most likely that signaling within the androgen receptor pathway drives prostate cancer cellular proliferation. Therefore AR over expression at baseline, via amplified gene expression or increased molecular stability, may be one mechanism of resistance.[9, 10]

Molecular changes in the AR such as AR splice variants could enhance ligand-independent AR signaling and lead to primary drug resistance.[6] Enhanced efficacy of intranuclear steroid receptors functioning downstream from the AR could serve as coactivators for transcription, promoting treatment resistance. In addition, prostate cancer cells may utilize autocrine- or paracrine-mediated cellular growth, producing various androgens to drive these aberrant mechanisms through cytochrome P17 (CYP17)–mediated androgen synthesis.[11, 12] Additional possible mechanisms include AR signaling cascades that have been associated with cellular proliferation and decreased apoptotic activity.[13]

1.2.4 Understanding Resistance Patterns

Broadly, several hypotheses may explain genetic resistance to ADT (**Table 1**):

I. Positive selection for intrinsic clonal or subclonal resistance effectors present within heterogeneous tumors,
II. Acquisition of new resistance effectors as a result of therapy, or
III. Adaptive cellular responses (e.g., feedback mediated up-regulation of bypass pathways).

Table 1. Potential resistance effectors and approaches for discovery.

Putative Resistance Mechanism	Sequencing Platform Discovery	Proposed Computational Solution
Tumor heterogeneity	Whole genome sequencing	Assess clonal and subclonal driver alteration selection
Selection for tumor suppressor losses or oncogene amplification	Whole genome	Assess for clonal and subclonal copy gains and losses
Acquisition of new variants	Whole genome sequencing	Identify acquired driver alterations
Adaptive cellular response	Transcriptome	Identify longitudinal alteration and expression changes

A goal of this proposal is to systematically interrogate these potential resistance mechanisms by computationally identifying in genomic resistance effectors to ADT in the preoperative setting. Previous studies have utilized massively parallel sequencing technologies to define the spectrum of somatic events occurring in primary and metastatic prostate cancer.

[14-16] These studies have identified multiple genomic events that may contribute to prostate oncogenesis.

1.2.5 Preliminary studies for prostate Multiparametric 3T prostate magnetic resonance imaging guided prostate biopsy

Recent literature supports the contention that Multiparametric 3T prostate magnetic resonance imaging (mpMRI) has high specificity for the detection of focal prostate cancer. [17] Multi-parametric techniques are a combination of morphologic and functional sequences. These mpMR exams include diffusion (DWI), contrast enhanced (DCE) and T2W sequences. This combination allows for a detailed assessment of focal cancers, based upon cellularity (DWI), perfusion (DCE), neovascularity (DCE) and cell density (T2W). Many studies have demonstrated the increased specificity for *in vivo* cancer detection, diagnosis, tumor volume and most importantly the ability to differentiate aggressive (Gleason 4 or greater) from the indolent (Gleason 3 or less) cancer. [17, 18] Each of the individual sequences T2W, diffusion (DWI) and dynamic contrast enhanced (DCE) have been tested and validated both individually and in combination. A recent study done here at the NCI reported positive predictive values of 98%, 98% and 100% for whole gland, peripheral zone and central gland lesions respectively and like other studies also suggest it is more accurate for focal lesions over 0.5 cm and for higher Gleason grade lesions.[17] As Turkbey et al have also shown, using ROC analysis the AUC value for MRI volume estimates of tumors over 0.5cm^3 is 0.95. [19]

1.2.6 MR-US prostate Biopsy

The MR-US "fusion" approach pioneered by Drs. Pinto and Choyke can be used to specifically biopsy lesions seen on mpMRI. Such targeted biopsies will be evaluated in the men prior to neoadjuvant anti-androgen therapy. All men will have a 3T MR examination and MR/US fusion guided biopsy will utilize custom biopsy probe with the embedded passive electromagnetic (EM) tracker that allows real-time tracking of the US image and needle location. 3D reconstructed US volume will be registered and fused with the pre-biopsy MRI dataset. Continuous correction of rigid motion between the reconstructed reference 3D US volume and real-time 2D US images will enable tracking of the target lesions over the course of the biopsy procedure. The technical aspects and validation of this approach have been described earlier.[20] Biopsy targets identified in the MRI and re-identified in TRUS by means of registration will be sampled under real-time US guidance. The EM tracking and fusion technology we plan to use for this procedure has been developed and thoroughly validated by the NCI, and has been applied in more than 195 cases. [21, 22]

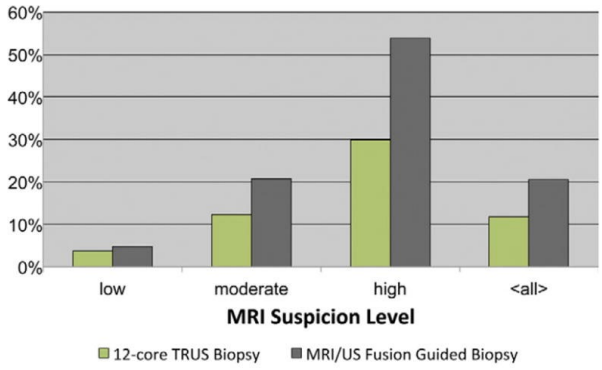
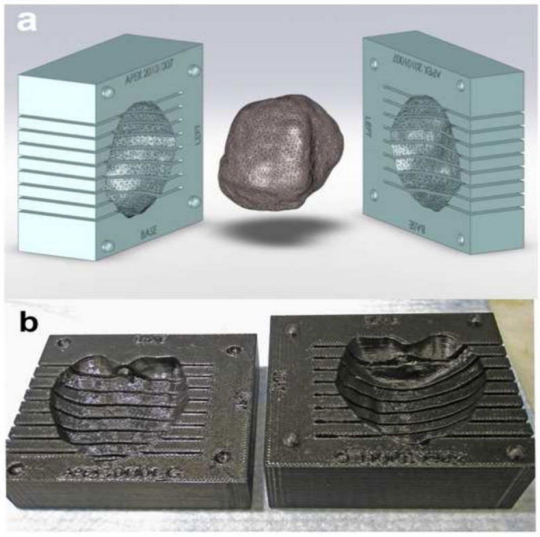


Figure 1: Cancer detection rates comparing 12 core TRUS and MR/US biopsy based upon MR suspicion level.

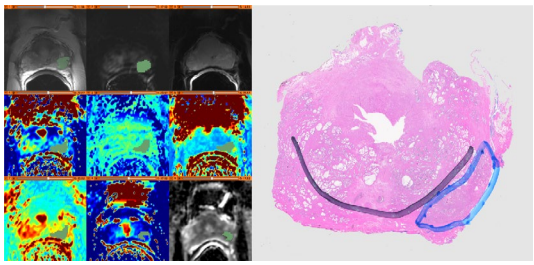
Custom made patient –specific MR based prostate gland molds: A 3D model of the prostate can be created in a novel technique developed and pioneered in the Clinical center (**Figure 2**), as described previously.[23] In brief it is a 3D model based upon MR images, which begins with manual segmentation of the MR T2W images. The prostate is outlined and converted via software into a 3D object. This is then imported into SolidWorks (SolidWorks, Dassault Systèmes SolidWorks Corp., Concord, MA, USA). There it is subtracted to create an internal cavity that precisely shapes the patients' gland. Slots for slicing the gland at 6mm intervals are designed and orientation markings (Superior/Inferior, Right/Left etc.) are added and it is then created using a 3D printer.

Figure 2: Customized, patient-specific prostate specimen mold. (a)Three-dimensional representation of final prostate mold in green, shown in two halves with a 3D model of the patient's prostate shown in brown. (b) Mold is created using a 3D printer, shown here in two halves fit together using small pegs/slots in the corners.



The mold approach has also been shown to allow for decision system support development [24] that provides a probability map of peripheral zone cancers based upon MRI. This expertise will be critical to the imaging, allowing suspicious lesions to be targeted for biopsy and subsequent correlative genetic analyses to be performed on the tissue from the index lesion(s). This unique tool allows for the most accurate alignment of the prostate specimen to MR images that is available today. This resource available here at the NCI will be essential for the success of this proposal allowing for precise validation of in vivo imaging studies by enabling registration for focal lesion core biopsy and whole mount histopathology.

Figure 3: Digitized multiparametric MRI maps (left) and the corresponding digitized whole-mount histopathology (right) slice with tumor outlined by a prostate cancer pathologist, and used as a landmark for lesion confirmation on mp MRI maps determine lesion localization.



1.2.7 Summary

Patients will be treated with goserelin and enzalutamide prior to RP. Learning what molecular profile is associated with resistance to intense anti-androgen therapy and linking this to predictive information from imaging will provide critical insight into the biology of prostate cancer and help develop predictive markers for potentially curative therapy. We will define the corresponding roles of imaging and genomics before and after treatment with goserelin and enzalutamide in men on this clinical trial

A complex but central prostate cancer research problem, the unpredictable resistance to ADT in high risk prostate cancer, is addressed in this study. There is currently no way to detect early resistance to ADT. As the common pathway for lethal prostate cancer is resistance to ADT, prediction and visualization of resistance to ADT through imaging and genomic markers would be a major advance for all men with this phenotype. It will allow quantification and characterization of the residual cancer burden (RCB) and this knowledge if predictive of long-term tumor response will allow real-time stratification of patients to the most effective therapy.

The advent of targeted therapies, exemplified by immunotherapy agents, creates new complexities in the assessment of response. There is increasing need for imaging modalities which can accurately and reproducibly measure not just change in tumor size, but changes in relevant metabolic parameters, modulation of relevant signaling pathways, drug delivery to tumor, and differentiation of apoptotic cell death from other changes in the tumor volume. Our studies here at the NCI have previously developed *three unique contributions* to the imaging and image guided diagnosis of prostate cancer, namely validated quantitative mpMR, MR-ultrasound guided biopsy techniques and mapping of tumor loci from MRI to prostatectomy specimen, by custom designed 3D molds.

ELIGIBILITY ASSESSMENT AND ENROLLMENT

1.3 ELIGIBILITY CRITERIA

1.3.1 Inclusion Criteria

1.3.1.1 Patients must have histologically or cytologically confirmed prostate cancer confirmed by the Laboratory of Pathology, NCI or Pathology Department at Walter Reed Bethesda.

1.3.1.2 Must have previously untreated (with definitive therapy) prostate cancer with intermediate or high risk features defined as:

Intermediate risk:

-PSA level is between 10 and 20 ng/ml or
-Gleason score is 7 or
-Stage T2b or T2c

High Risk:

-Gleason 8 and higher OR
-PSA > 20 at the time of diagnosis OR
-Seminal vesicle involvement OR
-Possible (on MRI) Extra-capsular extension (T3 disease)

1.3.1.3 Patients must be eligible for and must be planning to undergo radical prostatectomy

1.3.1.4 Patients must have testosterone levels ≥ 100 ng/dL

1.3.1.5 Men age ≥18 years.

Children are excluded because prostate cancer is not common in pediatric populations. Women are not eligible because this disease occurs only in men.

1.3.1.6 ECOG performance status ≤1 (see APPENDIX A-Performance Status Criteria).

1.3.1.7 Patients must have normal organ and marrow function as defined below:

– Hemoglobin	≥ 9 g/dL
– leukocytes	≥3,000/mcL
– absolute neutrophil count	≥1,500/mcL
– platelets	≥150,000/mcL
– total bilirubin	within normal institutional limits
– AST(SGOT)/ALT(SGPT)	≤3 X institutional upper limit of normal
– creatinine	within normal institutional limits
	OR
– creatinine clearance	≥60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal. (calculated via Cockcroft-Gault equation)

1.3.1.8 The effects of enzalutamide on the developing human fetus are unknown. For this reason and because androgen receptor antagonists as well as other therapeutic agents used in this trial are known to be teratogenic, male participants and their female partners of child bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence). Male participants should use a condom if having intercourse with a pregnant woman. Additionally, a condom plus another effective method of birth control is recommended during therapy and for 3 months after treatment for male participants having intercourse with a woman of reproductive potential. Should a woman become pregnant or suspect she is pregnant while her partner is participating in this study, she should inform her treating physician immediately.

1.3.1.9 Ability of subject to understand and the willingness to sign a written informed consent document.

1.3.1.10 Willingness to undergo biopsy.

1.3.1.11 Ability to detect lesions within prostate on MRI for biopsy

1.3.1.12 Willingness to travel to NIH for follow-up visits.

1.3.2 Exclusion Criteria

1.3.2.1 Patients who are receiving any other investigational agents (in the past 28 days) or herbal medications (within 1 day).

1.3.2.2 Patients with distant metastatic disease beyond N1 (regional) lymph nodes on conventional imaging studies (CT, MRI or Bone Scan).

1.3.2.3 Patients who have received any prior therapy for prostate cancer with surgery, radiation, and/or chemotherapy

1.3.2.4 History of allergic reactions attributed to compounds of similar chemical or biologic composition to enzalutamide or other agents used in study.

1.3.2.5 Clinically significant cardiac disease, e.g., New York Heart Association (NYHA) classes III-IV; uncontrolled angina, uncontrolled arrhythmia or uncontrolled hypertension, myocardial infarction in the previous 6 months as confirmed by an electrocardiogram (ECG).

1.3.2.6 Contraindication to biopsy:

- Bleeding disorders
- PT/PTT ≥ 1.5 times the upper limit of normal
- Artificial heart valve

1.3.2.7 Contraindication to MRI:

- Patients weighing more than weight limit for the scanner tables
- Allergy to MR contrast agent
- Patients with pacemakers, cerebral aneurysm clips, shrapnel injury or implantable electronic device

1.3.2.8 Uncontrolled intercurrent 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.

1.3.2.9 Patients with known HIV are ineligible. These patients are at increased risk of lethal infections when treated with marrow-suppressive therapy. In addition, if patients are receiving combination antiretroviral therapy, there is potential for pharmacokinetic interactions with enzalutamide. Appropriate studies will be undertaken in patients receiving combination antiretroviral therapy when indicated.

1.3.2.10 Patients with known active treatment for Hepatitis B and C infections.

1.3.2.11 Patients who are taking medications that are strong inhibitors of CYP3A4 or PgP and need to remain on these medications. For a current table of Substrates, Inhibitors and Inducers please access the following website:

http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm

1.3.2.12 History of seizure, including any febrile seizure, loss of consciousness, or transient ischemic attack, or any condition that may pre-dispose to seizure (e.g., prior stroke, brain arteriovenous malformation, head trauma with loss of consciousness requiring hospitalization).

1.3.2.13 Other medications used for urinary symptoms including 5-alpha reductase inhibitors (finasteride and dutasteride) and alternative medications known to alter PSA (e.g., phytoestrogens and saw palmetto) cannot be taken while patients are receiving enzalutamide

1.3.2.14 Patients with a malignancy within the past 3 years for which study drugs or a prostatectomy is a contraindication.

1.3.3 Recruitment Strategies

This protocol may be abstracted into a plain language announcement posted on NIH websites and on NIH social media platforms.

1.4 SCREENING EVALUATION

1.4.1 Screening activities performed after a consent for screening has been signed

Screening evaluation testing/procedures are conducted under the separate screening protocol, 01-C-0129 (Eligibility Screening and Tissue Procurement for the NIH Intramural Research Program Clinical Protocols).

- **Pathological confirmation** of malignancy by the Laboratory of Pathology, NCI or Pathology Department at Walter Reed National Military Medical Center.
- **Within 12 weeks** of study entry: Bone scan, CT and/or MRI scans as appropriate for disease evaluation.
- **Within 8 weeks** of study entry: ECG, laboratory tests: CBC with differential and platelet count, Creatinine, ALT, AST, total bilirubin, PT/PTT, serum PSA, testosterone level, focused history, physical examination with documentation of weight, measurable disease and performance status.
- **Within 6 months** of study entry: 3T mpMRI and U/S Guided Targeted Biopsy of the prostate

For baseline evaluations, please see Section **2.5**.

1.5 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

1.6 TREATMENT ASSIGNMENT PROCEDURES

Cohort

Number	Name	Description
1	Cohort 1	Men ≥18 years old with non-metastatic prostate cancer

Arm

Number	Name	Description
1	Arm 1	Patients will have an mpMRI guided biopsy, then receive enzalutamide and goserelin treatment for 6 months followed by a second mpMRI examination.

1.7 BASELINE EVALUATION

- History and physical examination within 17 days of treatment initiation (for medical record only).
- Laboratory and urine studies (Tests completed at screening may be omitted if performed *within 17 days* of treatment initiation): **CBC with differential** and platelet count, **PT/PTT**, and **Acute care panel** (Serum sodium, Potassium test, Serum chloride, CO2, Creatinine, Glucose test, BUN); **Mineral panel** (Albumin, Calcium, Magnesium, Phosphorus – serum); **Hepatic Panel** (Alkaline phosphatase, ALT, AST, direct and total bilirubin); Gamma-GT, LDH, total cholesterol, total protein, Serum PSA, uric acid, urinalysis
- Baseline ECG (within 17 days of treatment initiation)
- Vital signs including weight and height.

STUDY IMPLEMENTATION

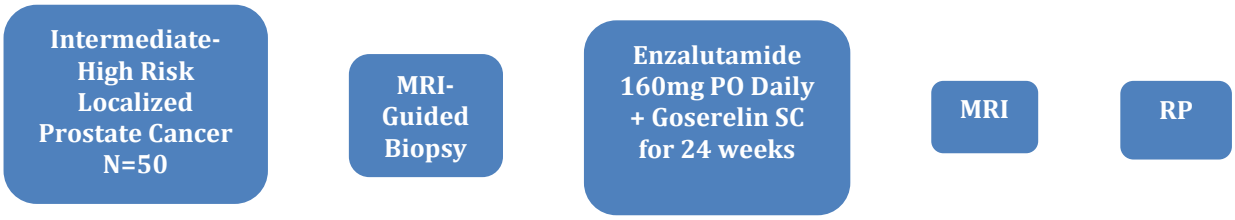
1.8 STUDY DESIGN

All men (n=50) enrolled in the trial will undergo two 3T mpMRI endorectal examinations, one at screening before treatment and a second after completing 6 months of goserelin and enzalutamide just prior to surgery. Radical Prostatectomy will be performed as per standard procedures at either the Clinical Center or Walter Reed Bethesda. Screening biopsy (MR/US guided) samples and tissue from the prostatectomy specimens will all undergo pathologic and genomic analysis. Genomic analysis will only be performed after obtaining informed consent. Genomic analysis will involve parallel sequencing and evaluation of molecular resistance mechanisms. We will obtain one or two cores from each index lesion(s)-up to 3 index lesions will be identified per patient and record all the needle locations. One core from each index lesion will undergo both routine pathologic examination as well as genetic analysis. Patients will be treated prior to surgery with standard doses of goserelin and enzalutamide. It is anticipated that different patients and conceivably different lesions within a patient may have differential responses to treatment by mpMRI. Genomic analysis will be performed from areas of

pathologically confirmed and MRI identified unresponsive areas of tumor within the same patient. In addition, using a whole-mount prostate mold, the same areas identified as were biopsied at screening (e.g., dominant lesion identified on mpMRI) can be directly re-biopsied for comparative genomic analysis.

Figure 4: Study Schema

After 6 months of goserelin and enzalutamide therapy, all men will have a second mpMRI examination. The primary goal being to create the 3D mold prior to prostatectomy and the secondary to obtain the post treatment mpMRI data.



1.9 Drug Administration

1.9.1 Enzalutamide

All patients will receive enzalutamide 160mg orally, daily for 24 weeks. Enzalutamide may be administered with or without food. The study drug will be dispensed by the institutional pharmacy.

1.9.2 Goserelin

Goserelin will be administered SC for a total of 24 weeks. The following are examples of acceptable regimens (no dose modifications will be permitted):

Goserelin 10.8mg administered subcutaneously every 12 weeks (2 doses).

(Equivalent forms of ADT would also be acceptable including other GnRH agonists/antagonists)

1.10 Dose Modifications

1.10.1 Enzalutamide Dose Modification

If a patient experiences a ≥ Grade 3 non-hematologic toxicity (except hypertension) attributable to enzalutamide or an intolerable side effect attributable to enzalutamide, withhold dosing for up to 1 month or until symptoms improve to ≤ Grade 2, then resume at the same dose if clinically appropriate. If toxicity recurs and or in the judgment of the investigator dose reduction is appropriate, lower dose levels (120 mg /DL-1 or 80 mg/DL-2), may be considered. Enzalutamide may be discontinued if toxicity recurs and dose modification is no longer clinically appropriate.

1.10.1.1 Enzalutamide Dose Delay

Enzalutamide may be started within 7 days if a dose is missed due to scheduling or logistical issues (e.g., vacation, weather) during the treatment period. If more than 7 days have elapsed, patients will need to be seen before further doses can be dispensed.

1.10.2 Enzalutamide Dose Modification for Hypertension

If a patient experiences hypertension attributable to enzalutamide, patients should be optimally managed prior to withholding enzalutamide dose. Clinical judgment should be used in deciding whether the new or worsened hypertension emerging during treatment with enzalutamide requires immediate therapy.

Grade 2 ≥ 140 mmHg (systolic) and < 160 mmHg OR ≥ 90 mmHg (diastolic) and < 100 mmHg	▪ Maintain dose of enzalutamide ▪ Increase antihypertensive therapy if clinically appropriate (consider increase dose of existing medications and/or add new antihypertensive medications
Grade 3 ≥ 160 mmHg (systolic) OR ≥ 100 mmHg (diastolic)	▪ Maintain dose of enzalutamide ▪ Increase antihypertensive therapy (increase dose of existing medications and/or add new antihypertensive medications) ▪ If optimal antihypertensive therapy does not result in a clinically significant reduction in blood pressure, enzalutamide should be held until blood pressure returns to baseline or < 140 / 90.
Grade 4	▪ Discontinue enzalutamide

1.11 IMAGING

1.11.1 mpMRI pre and post preoperative goserelin and enzalutamide

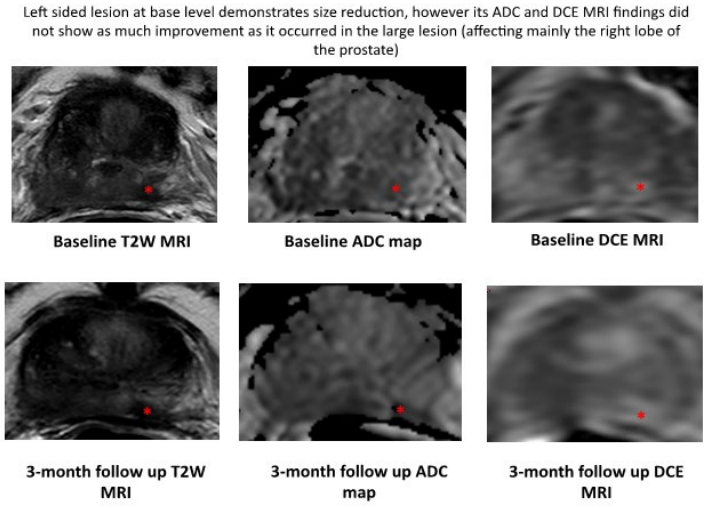
Patients will undergo pre-therapy ecoil MRI at 3T at screening. All MR data sets will be reviewed for quality and completeness. DCE PK analysis will be performed using commercially available iCAD software (http://www.icadmed.com). Post 6 months of preoperative therapy, a second ecoil MRI at 3T will be obtained. At both time points, quantitative T2, ADC values and DCE metrics will be extracted and recorded from all foci. DCE metrics to be collected include empirical indices such as Area Under the Curve at 90 seconds (AUC_{90}) and Time to Peak (TTP), and also the derived indices K^{trans} (the forward volume transfer constant of Gadolinium between blood plasma and the interstitial space, expressed in units of min^{-1}), and v_e, the dimensionless fractional volume of interstitial or extracellular space per unit volume of tissue. In addition, PiRads data will be collected for both time points.

1.11.2 Registration of pre- and post-therapy prostate MRIs

Drs. Choyke, Turkbey, and Harmon will collaborate on evaluation and registration of pre- and post-treatment MRIs. We will use non-rigid image registration for establishing correspondence between the targets and sub-areas in the baseline and post-therapy MR images. Preoperative therapies are known to significantly alter MR signal signatures for both normal and tumor tissue. [25, 26] We expect large reduction of the prostate gland from the baseline to post-treatment imaging session. These issues undoubtedly make the registration problem very challenging. Based on the limited preliminary evidence, intensity-based deformable registration based on Mutual information similarity metric pioneered by our group [27] can be used for co-registration of pre- and post-treatment prostate MRI. Our early preliminary results show the feasibility of such registration for the data collected in preoperative therapy cases (see **Figure 5** below). A

variety of deformable registration methods based on Mutual Information are available in 3D Slicer, [28] and will be applied to this problem. The transformations derived by the registration will be used to map the areas in the pre-treatment scans to the post-treatment gland configuration. This information will be used in manufacturing the custom mold that will include needle access to the image-registered locations of lesions to collect tissue samples from the ex vivo specimen.

Figure 5:



1.12 On–Treatment Evaluation

- Physical examination and vital signs
- Laboratory studies: **CBC with differential**, **PT/PTT**, **Acute care panel** (Serum sodium, Potassium test, Serum chloride, CO2, Creatinine, Glucose test, BUN); **Mineral panel** (Albumin, Calcium, Magnesium, Phosphorus – serum); **Hepatic Panel** (Alkaline phosphatase, ALT, AST, direct and total bilirubin); **LDH; Serum PSA; and Testosterone**
- Correlative studies (see Section **5.1**)

1.13 Follow up Procedures

After patient has completed their post-surgery visit, the patient will be contacted remotely (e.g., phone) annually up to five years by the research team to determine their PSA level. PSA levels may be obtained at NIH or through a local provider. If PSA recurs at 0.2 ng/mL or higher, the patient will come off study and will no longer be followed.

1.14 STUDY CALENDAR

Procedure	Screen	Base-line	Day 1[a]						Post-Tx --- Pre-Surgery	Post-Surgery[c]	Follow up[f]
			Month 1	*Month 2*	*Month 3*	*Month 4*	*Month 5*	*Month 6*			
Physical Exam & Vital signs	X	X	X	X	X	X	X	X	X	X	
Performance Score	X	X									
Labs (See Sections 2.2 and 2.5)	X	X	X	X	X	X	X	X	X	X	
PSA	X	X	X	X	X	X	X	X	X	X	X
Pathologic Confirmation	X										
Biopsy	X[e]										
3T mpMRI	X[e]								X		
Correlative Research Studies[b]		X				X			X	X	
Imaging studies[d]	X								X		
ECG	X	X							X		
Prostatectomy									X		
Enzalutamide			X	→	→	→	→	X			
ADT			X			X					
Adverse Events		X	→	→	→	→	→	→	X		
Concomitant Medications		X	→	→	→	→	→	→	X		
Telephone call											X

[a] Assessments will be performed on day one of each month of treatment ± 7 days
[b] See Section 5.1 for information on correlative research
[c] Approximately 12 weeks after surgery, may be in combination with urology branch post-surgical visit
[d] Imaging studies, if clinically indicated, for disease evaluation within 12 weeks of study entry and pre-prostatectomy
[e] Performed within 6 months prior to starting protocol therapy
[f] Follow-up will be at least annually and may be done remotely (see Section 3.6).

1.15 COST AND COMPENSATION

1.15.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures are performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

1.15.2 Compensation

Participants will not be compensated on this study.

1.15.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

1.16 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

1.16.1 Criteria for removal from protocol therapy

- Completion of protocol therapy
- Participant requests to be withdrawn from active therapy
- Unacceptable Toxicity (Section **3.3.1**).
- Investigator discretion

1.16.2 Off-Study Criteria

- Completed study follow-up period (Follow-up period will be 5 years or until PSA recurrence of 0.2 ng/mL whichever is sooner)
- Initiation of new therapy
- Participant requests to be withdrawn from study
- Death
- Study is cancelled

CONCOMITANT MEDICATIONS/MEASURES

Patients may be on concomitant drugs to prevent bone loss, including calcium, vitamin D, bisphosphonates and denosumab.

Other supportive care with blood components, antibiotics, analgesics, general medical therapy, etc., will be delivered as required.

1.16.3 General Guidelines

All medications (other than study drug) and significant non-drug therapies (including physical therapy and blood transfusions) taken within 28 days of starting study treatment through the 6 to 10 week safety follow up visit should be reported on the CRF.

While patients on protocol treatment, all medications required for the health of the patient are allowed with the following exceptions**:**

- Concurrent chemotherapy
- Concurrent radiation therapy
- Concurrent immunotherapy
- Concurrent anti-cancer radionuclides
- Concurrent systemic corticosteroid use (daily or every other day for continued use >**14 days**)
- Concomitant use of secondary hormonal treatments
- Concomitant use of herbal supplements

1.16.4 Seizure threshold lowering drugs

- Concomitant medications that can lower seizure threshold should be excluded during the course of this study, e.g., atypical antipsychotic drugs, phenothiazines, bupropion, aminophylline/theophylline, tricyclic and tetracyclic antidepressants, lithium, pimozide, venlafaxine.

1.16.5 Cytochrome P450 and P-glycoprotein

- Co-administration of enzalutamide with strong or moderate CYP2C8 inducers (e.g., rifampin) may alter the plasma exposure of enzalutamide. Moderate CYP2C8 inducers may be used concomitantly with enzalutamide at the investigator's discretion. If co-administration of enzalutamide with a strong CYP2C8 inhibitor cannot be avoided, reduce the dose of enzalutamide to 80 mg per day.
- Co-administration of enzalutamide with strong CYP3A4 inducers (e.g., carbamazepine, phenobarbital, phenytoin, rifabutin, rifampin, rifapentine) may decrease the plasma exposure of enzalutamide and should be avoided if possible. Selection of a concomitant medication with no or minimal CYP3A4 induction potential is recommended.
- Moderate CYP3A4 inducers (e.g., bosentan, efavirenz, etravirine, modafinil, nafcillin) and St. John's Wort may also reduce the plasma exposure of enzalutamide and should be avoided if possible.
- Concomitant use of enzalutamide with narrow therapeutic index drugs that are metabolized by CYP3A4 (e.g., alfentanil, cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus and tacrolimus), CYP2C9 (e.g., phenytoin, warfarin) should be avoided, as enzalutamide may decrease their exposure.
- Grapefruit, Seville oranges, and starfruit affect P450 and PgP activity. Concomitant use should be avoided.

CORRELATIVE STUDIES FOR RESEARCH

1.17 BIOSPECIMEN COLLECTION

Note: Tubes and media may be substituted based on availability with the permission of the PI or laboratory investigator.

1.17.1 Histopathological Analysis

After localization by MR of the desired pretreatment and post treatment biopsy sites, one or two immediately adjacent tissue cores will be obtained from the lesion. One core will be placed in formalin (FFPE) for processing for histopathology, to confirm tumor diagnosis, Gleason grade and extent of tumor. The second core, if obtainable (presumed to be nearly identical in features to the first core), will be reserved for molecular analyses detection of androgen receptor variants. This analysis will be done only if there is sufficient tissue and after obtaining informed consent.

Clinical protocol steps	**Procedures**	**Core pathology analyses**
Pre-treatment (MR 1): MRI Index lesion(s) identified	In vivo Biopsy Index lesion(s) Optional biopsy	#1: FFPE (histology) #1A FFPE & 1 H&E (molecular analyses) #2: Fresh frozen (optional)
ADP Therapy	Prostatectomy	Full pathology report issued
Post-treatment (MR 2): MRI Index lesion(s) identified (same lesion(s) as above)	Ex Vivo Biopsy Index lesion(s) Optional biopsy	#1: FFPE (histology) #1A FFPE & 1 H&E (molecular analyses) #2: Fresh frozen (optional)

Table 2: Clinical Protocol Procedures

At prostatectomy, the fresh prostate gland will be processed. The custom-made 3D mold, created for the individual patient with MRI data, will be used to guide placement of the "post treatment biopsy", which is actually a sampling of the prostatectomy specimen in the exact geometric location as the pre-treatment biopsy. The fresh prostatectomy specimen will be placed in the mold and the one or two core samples obtained will be handled as described above. Documentation of precise location of the biopsy will be recorded with appropriate coordinates, and location will be correlated with the subsequent cutting of tissue cross sections. Then, the tissue will be processed by NCI Laboratory of Pathology staff for diagnosis and pathology report generation. The cross sections will be labeled and will be cut into halves or quadrants for routine histology processing, while documenting which cross section and quadrant (right anterior #1, left posterior #1, etc.) from which they originated. This will serve as a guide to the location of the post treatment biopsy, as that location will have been recorded keeping in mind the cross section number. After the prostatectomy specimen has been signed out by the Pathology Department

with final diagnosis and report issued, correlation between the post treatment biopsy and the whole section will occur. The tissue section(s) from which the post treatment biopsy was taken will be analyzed morphologically and compared to the biopsy. If indicated, the block or blocks of the slides to be compared can be sectioned further for complete morphologic analysis of presence/absence of residual tumor, tumor grade, tumor extent, etc.

MR 2 data will be performed according to the specialized protocol reported previously. [17, 23] All tumor foci will be re-identified. The 3D mold will provide target localization and trajectory path for post prostatectomy core biopsies.

Prior to prostatectomy we will use the MRI data and resulting 3D custom built molds of the entire prostate gland and foci of tumor within it. [29] Focal tumors and all prior biopsy targets will be re-identified where possible and mapped on MR imaging. This allows for image-based targets and/or regions registered from baseline MR exam to be re-identified for repeat biopsy. This second set of biopsies will be taken after gland removal. The core needle biopsies will be taken before specimen fixation and pathological analysis. These cores will be taken from the pre-defined locations identified on MR and displayed in the 3D mold. Most importantly this will provide tissue samples from *the same location*s both before and after therapy and allow for sampling of sub regions of activity within the lesion based upon the mpMRI appearances. The prostate specimen can be whole mount sectioned in a customized mold, allowing geometric alignment to magnetic resonance imaging and therefore precise comparison of MRI/pathologic response. The prostate specimen, after the core needle biopsy samples have been removed, the specimen will be processed in the routine way with full histo-pathological and genomic analysis.

1.17.2 Immunohistochemistry

The Laboratory of Pathology and the Warren G. Magnuson Clinical Center will perform Immunohistochemistry on biopsied tissue, if the patient elects to have the procedure. Immunohistochemistry of these tissue specimens will be obtained for CD4, CD8, and FOX-P3. In addition, phenotypic analysis of infiltrating immune cells will be performed.

Immunohistologic grading schema of the lesions

Score	%positive cells of each subtype
0	0
1	1-25%
2	26-50%
3	>50%

All staining will be categorized as being membrane or nuclear.

The Laboratory of Genitourinary Cancer Pathogenesis will perform H&E and immunohistochemistry on radical prostatectomy specimens and biopsied tissue for research purposes. Immunohistochemistry will be performed on cases with treatment effect or residual tumor cells following neoadjuvant enzalutamide treatment to identify potential genes or proteins associated with resistance to therapy. Immunohistochemistry targets will include AR, AR-V7, PD-L1, PTEN, GR and MYC. Additional targets will be added upon evaluation of sequencing results (Section **5.1.3** below). Staining qualitative assessment and (semi-)quantitative

measurements will be performed in collaboration with Huihui Ye, M.D. at UCLA and Rosina Lis, M.D. at Dana-Farber Cancer Institute using scanned or glass slides. Slides will be coded, linked; Drs. Ye and Lis will not have access to PII.

1.17.3 Next-Generation Sequencing and Genomic Analysis

Prostate cancer genomics are markedly heterogeneous, even within an individual focus. Genomic heterogeneity is associated with therapy resistance in multiple studies, and it is hypothesized that existing subclones comprise subclonal resistance effectors. Alternatively, the acquisition of new genomic alterations may confer a resistance phenotype to tumor cell clones or subclones. The analysis of tumor genomics both before therapy and after completion of neoadjuvant therapy can elucidate molecular features of resistant effectors and indicate if they arose through enrichment of intrinsic heterogeneity or acquisition of new alterations. Whole genome and/or exome sequencing will be performed on DNA isolated from matched core biopsy samples (MR1.1a and MR 2.1a). DNA taken from the buffy coat (see section on ctDNA) or benign adjacent prostate tissue will be used for a reference sample from which somatic alterations are identified. Germline analysis of DNA for inherited alterations reveal valuable insight into mechanisms of disease and will only be used for this purpose. Additionally, DNA may be isolated from involved tissue in the prostatectomy specimen after completion of clinical diagnostic evaluation by the Laboratory of Pathology, as described above. DNA sequencing and genomic analysis will be done using approved protocols under the auspices of Drs. Sowalsky in the Laboratory for Genitourinary Cancer Pathogenesis, CCR.

Sample Collection

Pretreatment screening biopsy samples will be received by Laboratory of Pathology and processed in standard fashion using formalin fixation and paraffin embedding. Post-treatment biopsy samples will be obtained by Laboratory of Pathology and processed in standard fashion using formalin fixation and paraffin embedding, as described above. Biopsy samples and, if appropriate, adjacent areas of the prostatectomy specimen, will be used for molecular studies after completion of clinical diagnostic evaluation. To enrich for tumor cell purity, it is expected tumor specimens will undergo microdissection prior to isolation of DNA and preparation of sequencing libraries.

1.17.4 Circulating Tumor DNA (ctDNA)

Circulating tumor DNA (ctDNA) is shed into the bloodstream by cells undergoing apoptosis or necrosis and in patients a proportion of the fragmented DNA in circulation is derived directly from the tumor. In recent years, ctDNA has shown promise as a non-invasive sensitive biomarker of tumor burden, and hence has potential as both a prognostic and surrogate response biomarker. In a study to detect ctDNA across 14 tumor types that had not yet metastasized or released detectable circulating tumor cells, ctDNA was detected at relatively high concentrations in the patients with metastatic cancer and at lower but detectable concentrations in those with localized cancers.[30] Together, these findings suggest that ctDNA could be a reliable biomarker for early detection as well as for determining optimal treatment, and monitoring resistance. ctDNA is abundant and readily detectable in most patients with advanced cancer; in cases with lower levels of ctDNA such as in early stage disease or minimal residual disease, detection is made possible with the use of advanced genomic methodologies. The analysis of ctDNA has considerable potential in the assessment of molecular heterogeneity, for monitoring

tumor dynamics, identifying genetic determinants of therapy, and evaluating treatment response; as well as detecting acquired resistance and developing potential strategies to circumvent this.[31] A recent study found that genomic analysis of ctDNA in plasma of mCRPC patients treated with abiraterone and enzalutamide identified key aberrations that may be associated with therapeutic resistance.[32] This data will be for research purposes only and will not be shared with patients.

Sample Collection

A baseline blood sample will be collected at the start of study and serial blood samples will be collected during the course of treatment at q3months, and at the end of study. Blood samples will be collected in 2 10-ml Streck Cell-Free DNA tube at room temperature and delivered to Dr. Figg's lab for barcode labeling and/or processing and then Bldg. 37, Rm1066 by Clinical Center Messenger Escort Service using a CRIS order. Processing and/or sequencing of plasma for ctDNA analysis will be performed by the Laboratory of Genitourinary Cancer Pathogenesis (Dr. Sowalsky).

1.17.5 Androgen Receptor Variants

Detection of androgen receptor variants in tissue samples will be performed on one-half of one core biopsy to isolate total RNA from screening biopsy and radical prostatectomy specimens. RNA will be reverse transcribed and full-length androgen receptor (AR with LBD) as well as androgen receptor variants 5, 6, 7, 8 (V5-V8) will be detected by droplet digital PCR. This analysis will be performed when logistically feasible and only if there is sufficient tissue available.

Sample Collection

A baseline and post-treatment tumor sample will be collected, if feasible. Notify Jane Trepel's lab in advance of the scheduled procedure by email. At the time of the procedure, please notify Ms. Trepel's lab by phone at 301-496-1547. A member of Ms. Trepel's lab will be present for the procedure to collect the sample, if available. Ms. Trepel's lab will provide the collection media.

1.17.6 Peripheral Blood microRNA and mRNA

MicroRNA will be isolated from plasma and analyzed using Exiqon microRNA PCR panels. AR full-length (with the LBD) and splice variants will be assayed from CTCs isolated from peripheral blood drawn into two 10-ml lavender top tubes and from RNA purified from blood collected in one 2.5-ml PAXgene tube. The copy number of splice variants will be quantified using droplet digital PCR and a comparison performed between PAXgene and CTC-enriched samples. This sample will be collected only when logistically feasible

Sample Collection

For microRNA analysis, peripheral blood will be collected in two 10-ml EDTA lavender top tubes and one 2.5-ml PAXgene tube at baseline and post-treatment, if feasible. Blood samples will be held at room temperature and picked up by Ms. Trepel's lab. Contact study team for PAXgene tube.

1.17.7 Tumor Gene Expression

In addition to acquiring resistance through enrichment or acquisition of genomic alterations, resistance may also arise through adaptive cellular responses in the absence of identifiable genomic alterations. This can best be evaluated through evaluation of tumor gene expression. Total RNA will be isolated from matched tumor tissue both before and after therapy, reverse transcribed, and used for transcriptome analysis. RNA will be isolated from matched FFPE cores (MR 1.1a and MR 2.1a). Additionally, RNA may be isolated from involved tissue in the prostatectomy specimen after completion of clinical diagnostic evaluation by the Laboratory of Pathology, as described above. Transcriptome sequencing and analysis will be done using approved protocols under the auspices of Dr. Sowalsky in the Laboratory for Genitourinary Cancer Pathogenesis, CCR.

Sample Collection

Pretreatment screening biopsy samples will be received by Laboratory of Pathology and processed in standard fashion using formalin fixation and paraffin embedding. Post-treatment biopsy samples will be obtained by Laboratory of Pathology and processed in standard fashion using formalin fixation and paraffin embedding, as described above. Biopsy samples and, if appropriate, adjacent areas of the prostatectomy specimen, will be used for molecular studies after completion of clinical diagnostic evaluation. To enrich for tumor cell purity, it is expected tumor specimens will undergo microdissection prior to isolation of RNA and preparation of sequencing libraries.

1.17.8 Management of Results

The results of molecular studies conducted using specimens collected on this protocol are for research purposes only and will not be disclosed to individual subjects. The exception to this is potential incidental findings that are deemed medically significant and actionable. The policy for this disclosure is outlined below and in the consent form for this study.

Subjects will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. (A list of current guidelines is maintained on the CCR intranet: https://ccrod.cancer.gov/confluence/display/CCRCRO/Incidental+Findings+Lists). Inherited alterations that may contribute to disease susceptibility in addition to prostate cancer may also be uncovered. Subjects will be contacted at this time with a request to provide a blood sample to be sent to a CLIA certified laboratory. If the research findings are verified in the CLIA certified lab, the subject will be offered the opportunity to come to NIH (at our expense) to have genetic education and counseling to explain this result. If the subject does not want to come to NIH, a referral to a local genetic healthcare provider will be provided (at their expense).

This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

Subject's genetic data may be deposited in a database such as dbGaP. Although there is controlled access to such a database, such a submission carries theoretical risks of revealing the identity of the subject. This is discussed in the consent.

1.17.9 Comparison of pathology to imaging

The use of a patient-specific mold (as described in Section **1.2.6.**) allows the radical prostatectomy specimen will be sectioned in the same plane as the MRI scans (see Section **3.4.1** and **3.4.2**), and thus any immunohistochemistry (IHC) performed (see Sections **5.1.1** and **5.1.2**) may help to inform analysis or interpretation of MRI in the post-treatment setting. There are no commercial software packages that specifically address the issue of automated quantification of post-treatment IHC and alignment with MRI. Drs. Choyke, Turkbey, and Harmon in the Molecular Imaging Program will collaborate with Drs. Sowalsky in the Laboratory of Genitourinary Cancer Pathogenesis to identify appropriate programs and tools for assessing correlation. As necessary, collaborations with third parties (including academic centers and commercial manufacturers of quantitative software) may be sought for finding the optimal solution. If a collaboration with an external party is to be sought, a personnel change amendment to this protocol will be made. All data shared with third parties will be coded, linked and covered under a human-materials MTA written specifically for each collaboration (see Section **9.1**).

1.18 Sample Storage, Tracking and Disposition

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH appropriate approvals and/or agreements, if required.

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described below. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed. The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of Section **7.2**.

1.18.1 Sample Data Collection - Blood Processing Core (BPC)

Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

All samples sent to the Blood Processing Core (BPC) will be barcoded, with data entered and stored in the Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined Figg lab personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen.
Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without Labmatrix access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center

patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

1.18.2 Sample Storage and Destruction

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed. The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per requirements of Section **7.2.1**.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the Labmatrix. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

DATA COLLECTION AND EVALUATION

1.19 DATA COLLECTION

The PI will be responsible for overseeing entry of data into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first study intervention, Study Day 1, through the end of treatment. Beyond 30 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

End of study procedures: Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

The NCI investigators will be responsible for the collection, maintenance, and quality control of the study data. Clinical data will be entered into a data capture system provided by the NCI CCR at least once every two weeks when patients are enrolled on the trial. Protocol-specific eCRFs will be developed for this trial. All data will be kept secure.

Only the following adverse events will be recorded on the Case Report Form:

- Grade 2 unexpected events that are possibly, probably or definitely related to the study drugs;
- Grade 3 and 4 events;
- Grade 5 events regardless of attribution;
- Serious Events regardless of attribution;
- Surgical side effects will be collected at the post-surgery visit only and not recorded if they occur after that time.

1.20 RESPONSE CRITERIA

For the purposes of this study, patients will be monitored for response via endorectal MRI for research purposes, but there will be no formal radiographic assessment of clinical response. It is anticipated that all patients will have a stable or declining PSA throughout the study (prior to surgery) and thus additional response assessments are not required.

Index lesions identified on mpMRI will be entered into a data capture system provided by the NCI CCR.

In the rare case that patients develop a rising PSA while on this study appropriate imaging studies will be performed at the discretion of the treating physician.

Definitions:

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with enzalutamide.

Evaluable for objective response: As described in the first paragraph above, patients will be followed for PSA and changes on endorectal MRI for research purposes only.

1.20.1 Disease Parameters

All patients will be required to have pathologically proven prostate cancer at enrollment and confirmation that they do not have metastatic disease.

1.21 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

NIH REPORTING REQUIREMENTS / DATA AND SAFETY MONITORING PLAN

1.22 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

1.23 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

1.23.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

1.23.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

1.24 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reviewed by the OHSRP in the NIH eIRB system will also be reported to the NCI Clinical Director/designee; therefore, a separate submission for these reports is not necessary.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to NCICCRQA@mail.nih.gov within one business day of learning of the death.

1.25 NIH Required Data and Safety Monitoring Plan

1.25.1 Principal Investigator/Research Team

The clinical research team will meet weekly when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in Section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

STATISTICAL CONSIDERATIONS

The primary objective of this exploratory trial is to obtain pilot data to assess the utility and role of mpMRI in localization and detection of focal prostate cancer both before and after treatment with pre-operative ADT and enzalutamide. Secondary objectives include obtaining pilot data to guide future studies with respect to determining genetic predictors and correlates of response to therapy as well as to link imaging information to genomic information related to resistance.

Patients who have newly diagnosed prostate cancer will undergo treatment with androgen deprivation therapy and enzalutamide and will undergo mpMRI imaging at screening and 6 months. The imaging will be used to monitor the changes in the individual tumors located within the prostate. As these patients will all have multiple tumors within the prostate, it is anticipated that approximately 25% of the patients will experience minimal declines in tumor and still have significant residual disease, 25% may have complete disappearance of tumor, and the remainder will show substantial improvement (disappearance of some lesions, with others being much smaller). To guide sample size determination, it is estimated that for an exploratory investigation of the association of genomic information and its relationship to response, it would be useful to have at least a minimum of 10 patients who still have significant residual disease in order to form preliminary assessments of the relationships noted. As such, if 50 total evaluable patients were to enroll on the trial and if the probability of being in this category was 25%, there would be 83.6% probability of having at least 10 such patients. Thus, this pilot study will plan to enroll 50 total evaluable patients who would all undergo primary treatment and mpMRI imaging to assess the role of imaging in this setting. The accrual ceiling will be 55 in order to accommodate a small number of patients who may be inevaluable. Successful use of this imaging will lead to analyses related to the association with genomic traits at baseline and prostatectomy and whether there is disappearance of tumor or not. On this trial, all of these investigations will be performed with exploratory, hypothesis generating intent.

HUMAN SUBJECTS PROTECTIONS

1.26 Rationale For Subject Selection

1.26.1 Selection Based on Ethnicity, and Race

Subjects from all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one group compared with another. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on one hand and the need to explore ethnic aspects of clinical research on the other hand. If differences in outcome that correlate with ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully. Women are not eligible for this protocol as this disease occurs only in men.

1.27 Participation of Children

Because no dosing or adverse event data are currently available on the use enzalutamide in patients <18 years of age and because prostate cancer is uncommon in pediatric populations, children are excluded from this study, but may be eligible for future pediatric trials.

1.28 Participation of Subjects Unable to Give Consent

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (Section **9.5**), all subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation to assess ongoing capacity of the subjects and to identify an LAR, as needed.

Please see Section **9.6.1** for consent procedure

1.29 Evaluation of Benefits and Risks/Discomforts

1.29.1 Alternative Approaches or Treatments

Patients will be consented regarding the risks and benefits of this trial, the treatment requirements, and alternative approaches to entering on this trial.

1.29.2 Identified Risks to Study Procedures

1.29.2.1 Blood Sampling

Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting.

1.29.2.2 Urine Collection

No physical risks are associated with urine collection.

1.29.2.3 Electrocardiogram

Some skin irritation can occur where the ECG/EKG electrodes are placed. The test is completely painless, and generally takes less than a minute to perform.

1.29.2.4 Tumor Biopsy

All care will be taken to minimize risks that may be incurred by tumor sampling. However, there are procedure-related risks (such as bleeding, infection and visceral injury) that will be explained fully during informed consent. The patient will consent at the time of the procedure.

1.29.2.5 MR Imaging

The risks of MR imaging are relatively small.

The US Food and Drug Administration has issued warnings that administration of gadolinium (updated September 9, 2010), the MRI contrast imaging agent used in this protocol, has been associated with development of a disease called **nephrogenic systemic fibrosis (NSF)**. The syndrome is rare (approximately 600 cases reported worldwide as of September 2010, out of several million administrations of gadolinium), but disabling and in some cases, fatal. All cases to date have occurred in patients with severe renal disease, including patients on dialysis. NSF has been nearly eradicated secondary to careful screening of renal function and avoiding use of gadolinium in patients with eGFR <30 ml/min/1.73 BSA. Even in patients with end stage renal disease, there have been only rare occurrences of NSF because of precautions taken to use more stable contrast agents at lower doses. This protocol excludes patients with severe renal insufficiency (eGFR <30 ml/min/1.73 BSA). The FDA has issued warnings in 2017 and 2018 that some gadolinium may be retained in the brain, bone and skin although health risks of accumulation have not been reported to date. In accordance with the FDA Drug Safety Communication of 05/16/2018, the Medication Guide for gadobutrol (or other macrocyclic gadolinium contrast agent if applicable) will be made available to all subjects with scans that will involve gadolinium-based contrast agent administration.

1.29.3 Non-Physical Risks of Genetic Research

1.29.3.1 Risk related to possibility that information may be released

This includes the risk that data related to your genetic testing can be released to members of the public, insurers, employers, or law enforcement agencies.

1.29.3.2 Risk of receiving unwanted information

Anxiety and stress may arise as a result of the anticipation that unwanted information regarding disease related genetic analysis. Patients will be clearly informed that the data related to genetic analysis is coded, investigational and will not be shared with patients, family members or health care providers.

1.29.3.3 Risk to family or relatives

Family members or relatives may or may not want to be aware of familial tendencies or genetic risks of disease which may cause anxiety about possible future health problems. As previously noted, patients will be notified of any medically significant and actionable incidental findings. Study results will not be shared with patients.

1.29.4 Procedure for Protecting Against or Minimizing any Potential Risks

All care will be taken to minimize side effects, but they can be unpredictable in nature and severity. This study may involve risks to patients, which are currently unforeseeable. All patients will have blood tests, examinations and scans as described in the protocol evaluation (Section **3.7**, Study Calendar). Patients will also be required to have a local physician to provide long-term care and to monitor for complications. If patients suffer any physical injury as a result of the participation in this study, immediate medical treatment is available at the Clinical Center, National Cancer Institute, Bethesda, Maryland. Although no compensation is available, any injury will be evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations.

1.29.5 Provisions for Monitoring Data Collection to Ensure Safety of Subjects

As information is gathered from this trial, clinical results will be shared with patients as they become available. Laboratory and clinical data will be frequently gathered and any new significant finding(s) found during the course of the research, which may affect a patient's willingness to participate further, will be explained. Confidentiality of information concerning participants will be maintained, including in all publications and presentations resulting from this study. Names of participants and/or material identifying participants will not be released without permission, except as such release is required by law. Records at the National Cancer Institute are maintained according to current legal requirements, and are made available for review, as required by the Food and Drug Administration or other authorized users, only under the guidelines established by the Federal Privacy Act.

1.30 Risks/Benefits Analysis

The risks of blood draw, MR imaging, and biopsy are relatively small. Because prostate biopsy is a clinically accepted intervention in patients with no evidence of metastatic disease, the risk of this study does not significantly differ from the risk of standard therapy with the exception that MR guided prostate biopsies typically involve obtaining a greater number of biopsies than a standard. In addition, the drug (dosage and formulation) being used in this study has been approved by the FDA for the treatment of prostate cancer.

1.31 Consent Process and Documentation

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is

conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When required, witness signature will be obtained similarly as described for the investigator and participant.

Manual (non-electronic) signature on electronic document:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location but is not required.

Both the investigator and the participant will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

1.31.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in Section **9.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **9.6**.

REGULATORY AND OPERATIONAL CONSIDERATIONS

1.32 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants and the Institutional Review Board (IRB) and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met

- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the IRB.

1.33 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Council on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

1.34 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Cancer Institute has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

1.35 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, and their staff. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party.

All research activities will be conducted in as private a setting as possible.

The study monitor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the/each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB or Institutional policies..

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site(s) and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

PHARMACEUTICAL INFORMATION

1.36 ENZALUTAMIDE

Please see FDA-approved packet insert for Enzalutamide for complete agent information.

1.36.1 Source

Enzalutamide will be obtained from commercial sources and dispensed by the institutional pharmacy.

1.36.2 Administration Procedures

Enzalutamide 160 mg (four 40 mg capsules) administered orally once daily.

Swallow capsules whole. Enzalutamide can be taken with or without food.

1.37 GOSERELIN

Please see package insert for complete drug information.

1.37.1 Source

Goserelin will be obtained from commercial sources and dispensed by the institutional pharmacy.

1.37.2 Administration Procedures

See Section **3.2.2** for details.

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APPENDIX A-PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	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.