UT SOUTHWESTERN
Harold C. Simmons Cancer Center

Phase II study of enzalutamide (MDV3100) and gonadotropin-releasing hormone (GnRH) agonist before, during, and after radiation therapy in treatment of patients with high-risk localized prostate cancer

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Signature Page

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

Principal Investigator (PI) Name:_____

PI Signature: _____

Date:_____

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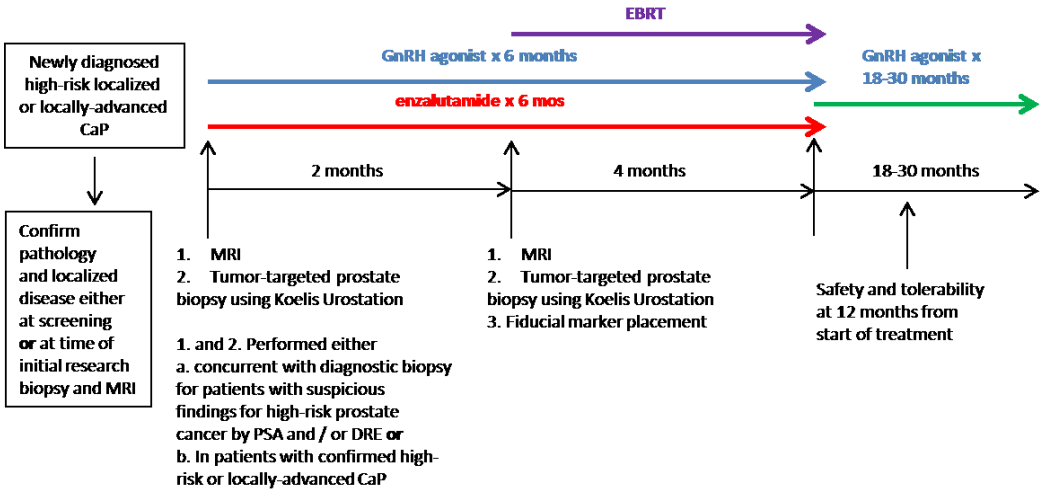
LIST OF ABBREVIATIONS

Examples Include:

3D-CRT	Three Dimensional Conformal Radiation Therapy
ADC	Apparent Diffusion Coefficient
ADT	Androgen Deprivation Therapy
AE	Adverse Event
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AR	Androgen Receptor
AST	Aspartate Aminotransferase
AUC	Area Under Curve
BUN	Blood Urea Nitrogen
CaP	Prostatic Adenocarcinoma
CBC	Complete Blood Count
CLIA	Clinical Laboratory Improvement Amendments
CMP	Comprehensive Metabolic Panel
CRPC	Castrate-Resistant Prostate Cancer
CT	Computed Tomography
CTV	Clinical Target Volume
CTC	Common Toxicity Criteria
CTCAE	Common Toxicity Criteria for Adverse Events
DHEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
DSMB	Data and Safety Monitoring Board
EBRT	External Beam Radiation Therapy
ECOG	Eastern Cooperative Oncology Group
eGFR	Estimated Glomerular Filtration Rate
EORTC	European Organization for Research and Treatment of Cancer
FDA	Federal Drug Administration
GnRH	Gonadotropin Releasing Hormone
GTV	Gross Tumor Volume
H&P	History & Physical Exam
HRPP	Human Research Protections Program
IC50	Inhibitory Concentration 50%
IMRT	Intensity-Modulated Radiation Therapy
IRB	Institutional Review Board
IV (or iv)	Intravenously
kV	Kilovoltage
LDH	Lactate Dehydrogenase
MG (or mg)	Milligram

ML (or mL)	Milliliter
MRI	Magnetic Resonance Imaging
MV	Megavoltage
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NG (or ng)	Nanogram
PK	Pharmacokinetics
PSA	Prostate Specific Antigen
PD	Progressive Disease
PFS	Progression Free Survival
p.o.	peros/by mouth/orally
PR	Partial Response
PTV	Planning Target Volume
qRT-PCR	Quantitative Reverse Transcription Polymerase Chain Reaction
RTOG-ASTRO	Radiation Therapy Oncology Group – American Society for Therapeutic Radiology and Oncology
SAE	Serious Adverse Event
SD	Stable Disease
SGOT	Serum Glutamic Oxaloacetic Transaminase
SPGT	Serum Glutamic Pyruvic Transaminase
T1/2 (or t1/2)	Half-life
TRUS	Transrectal Ultrasound
WBC	White Blood Cells

STUDY SCHEMA



STUDY SUMMARY

Title	Phase II pilot study of enzalutamide (MDV3100) and gonadotropin-releasing hormone (GnRH) agonist before, during, and after radiation therapy in treatment of patients with high-risk localized prostate cancer
Short Title	Phase II trial of radiation with enzalutamide (MDV3100) and androgen deprivation
Protocol Number	The standard protocol number used to identify this study
Phase	2
Methodology	Open label
Study Duration	5 years
Study Center(s)	Single-center
Objectives	This is a single institution phase II trial studying the safety and efficacy of combined enzalutamide and GnRH agonist therapy administered with EBRT for high-risk and locally advanced prostate cancer.
Number of Subjects	7

Diagnosis and Main Inclusion Criteria	- Patients must be candidates for long-term androgen deprivation in combination with EBRT for the treatment of high-risk or locally-advanced prostate cancer by the following criteria: o High risk disease: T3a or Gleason 8-10 or serum PSA > 20 ng/mL ▪ Gleason 7 also allowed if > 50% of cores positive for cancer or PSA velocity > 2 ng/mL/year in preceding 12 months o Locally advanced (very high risk) disease: T3b-T4 - - Patients may have radiographic evidence of metastasis in regional lymph nodes (N1 disease as defined by the National Comprehensive Cancer Network Prostate Cancer Guideline Verson 3.2012) at the discretion of the treating physicians, if regional lymph nodes can be included in the planned radiation field.
Study Product(s), Dose, Route, Regimen	Enzalutamide (MDV3100) (Xtandi), 4 x 40 mgcapsules (160 mg total) by mouth daily
Duration of administration	6 months
Reference therapy	N/A
Statistical Methodology	Descriptive statistics will be used in the analysis of safety and tolerability and the outlined imaging and biological correlates.

1.0 BACKGROUND AND RATIONALE

1.1 Disease Background

Androgens fuel the growth of prostate cancer cells. Interfering with androgen signaling impairs prostate cancer growth. External beam radiation therapy (EBRT) employs x-rays to kill tumor cells and is an effective treatment for localized prostate cancer. Previous clinical trials have demonstrated that combining EBRT with inhibitors of androgen signaling improves overall survival in patients with high-risk and locally-advanced prostate cancer.[1-9] D'Amico and colleagues reported that 6 months of combined androgen suppression therapy by administration of a GnRH agonist and an anti-androgen before, during, and after EBRT improved survival compared to EBRT alone in patients with intermediate-risk or high-risk prostate cancer.[4] RTOG 9202 was a randomized study of 1521 patients treated with radiation therapy plus 4 months of the GnRH agonist goserelin with the anti-androgen flutamide before and during radiation therapy, followed by 0 vs 24 months of goserelin.[5] The study showed an improvement in disease free survival at 10 years. Overall survival showed no statistically significant difference between the two arms, but subgroup analysis showed an overall survival benefit for patients with prostatic adenocarcinoma of Gleason score 8-10.[5] In the phase III randomized trial EORTC 22961, Bolla and colleagues subsequently reported that 3 years of androgen suppression therapy (comprised of 6 months of combined GnRH agonist and androgen receptor antagonist treatment followed by 30 months of GnRH agonist therapy) before, during, and after EBRT lead to improved overall survival compared to 6 months of combined androgen suppression therapy plus EBRT in patients with high-risk prostate cancer.[2] Taken together, these data provide category 1 evidence for treatment of high-risk and locally advanced prostate cancer with EBRT plus long-term neoadjuvant / concomitant / adjuvant androgen suppression therapy for 2-3 years as a standard of care (www.nccn.org). However, a substantial proportion of these patients will develop metastatic prostate cancer and die as a result of their metastatic disease. We propose that enzalutamide plus GnRH agonist treatment in combination with EBRT should be safe and well tolerated for the treatment of prostate cancer. We propose to carry out a pilot phase II study to assess the safety and tolerability of this combination, along with imaging and biologic correlates to assess response to enzalutamide plus GnRH treatment prior to initiation of EBRT. We hypothesize that enzalutamide plus GnRH agonist combined with EBRT may improve survival compared to standard treatment with GnRH agonist plus first-generation anti-androgens combined with EBRT.

1.2 Study Agent(s) Background and Associated Known Toxicities

Enzalutamide (MDV3100, Xtandi) is an androgen receptor inhibitor. In August 2012, enzalutamide received US FDA approval for the treatment of patients with metastatic castration-resistant prostate cancer who have previously received docetaxel. The approved dose of enzalutamide is 160 mg (four 40 mg capsules) by mouth once daily.

Although the primary endpoint of this study is to assess the safety and tolerability of enzalutamide plus GnRH agonist combined with EBRT, there is no reason a priori to believe that this combination of therapy will increase the known potential risks associated with enzalutamide. There may be unexpected risks from this combination, and this possibility will be evaluated in the clinical study.

We believe that this study meets criteria for exemption from application for an Investigational New Drug (IND) based on the following criteria as enumerated in the Code of Federal Regulations (CFR 312.2) and copied directly below:

“Exemptions. (1) The clinical investigation of a drug product that is lawfully marketed in the United States is exempt from the requirements of this part if all the following apply:

(i) The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication for use nor intended to be used to support any other significant change in the labeling for the drug;

(ii) If the drug that is undergoing investigation is lawfully marketed as a prescription drug product, the investigation is not intended to support a significant change in the advertising for the product;

(iii) The investigation does not involve a route of administration or dosage level or use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product;

(iv) The investigation is conducted in compliance with the requirements for institutional review set forth in part 56 and with the requirements for informed consent set forth in part 50; and

(v) The investigation is conducted in compliance with the requirements of 312.7.” (http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.2)

The presented background information about the study agent enzalutamide (MDV3100, Xtandi) is taken directly from the Enzalutamide Investigators Brochure provided by Astaellas Pharma U.S., Inc.: Medivation, Inc. MDV3100 Investigator's Brochure 23 MAR 2012 - v5.0 FINAL. More information is provided in the Investigator’s Brochure.

1.2.1 Summary of Pre-Clinical Studies of enzalutamide (MDV3100):

Enzalutamide (MDV3100) is an androgen receptor (AR) signaling inhibitor rationally designed to block multiple steps in the AR signaling pathway and to be devoid of agonist activity. MDV3100 inhibits androgen-induced receptor activation (binding of androgens to ARs in the cytosol), inhibits nuclear translocation of activated ARs, and inhibits the association of the activated AR with chromatin, even in the setting of AR overexpression and in prostate cancer cells resistant to anti-androgens such as bicalutamide. The consequence of MDV3100 AR signaling inhibition is decreased growth of prostate cancer cells, induction of cancer cell death, and tumor regression.

Safety pharmacology studies in mice, rats, and dogs were performed with MDV3100 to assess any acute effects on central nervous system, respiratory, and cardiovascular parameters. No MDV3100-related effects were noted in the central nervous system study assessing a functional observation battery and in a respiratory study in rats. MDV3100 inhibits the human ether-a-go-go-related gene (hERG) channel; however, the highest free concentrations of MDV3100 expected in patient plasma at a steady-state dose of 160 mg/day are well below the hERG inhibitory concentration 50% (IC50) value. No MDV3100-related effects on cardiac electrophysiology were noted in a safety pharmacology study in conscious, telemetered dogs.

As MDV3100 inhibits the gamma amino butyric acid (GABA)-gated chloride channel, convulsion potential was assessed in single- and multiple-dose studies in mice.5 Oral MDV3100 daily for 7 days was associated with convulsions in a dose-dependent manner with doses ≥ 200 mg/kg being active. When administered as a single dose of 400 mg/kg MDV3100 treatment was also associated with convulsions. At the highest dose at which no convulsions occurred (single dose, 100 mg/kg), the maximum plasma concentration

(Cmax) and area under the curve at 24 hours after dosing (AUC24) were at least 2.5-times higher than those in patients receiving 160 mg/day.

MDV3100 is metabolized primarily to 2 metabolites, M1 (a carboxylic acid derivative) and M2 (N-desmethyl MDV3100). The metabolite M2 has a pharmacology profile similar to that of the parent molecule and may contribute to the therapeutic effects of MDV3100 in patients. M2 also binds to and inhibits the GABA-gated chloride channel with potency similar to that of the parent molecule and may contribute to convulsion risk. Metabolite M1 does not have significant pharmacological actions and probably does not contribute to the therapeutic effects of MDV3100.

Nonclinical Pharmacokinetics and Metabolism
Following oral administration, MDV3100 has a half-life (t1/2) of approximately 0.25 to 3 days in mice, rats, dogs, and monkeys. The t1/2 does not appear to be affected by the dose size; however, the bioavailability in animals appears to decrease with increasing dose size.
In vitro studies show the following:
・MDV3100 is metabolized by human recombinant cytochrome P450 (CYP) isoenzymes CYP2C8 and CYP3A4/5;
・MDV3100 and/or its major human metabolites are potential inhibitors of CYP2C8 and CYP2C19 with lesser potential for inhibitory effects on CYP2B6 and CYP2C9;
・MDV3100 is a potential inducer of CYP3A4;
・MDV3100 is a potential inhibitor, but not a substrate, of the efflux transporter permeability glycoprotein (P-gp);
・The protein binding of MDV3100 in human plasma is 97% to 98% and is similar in mice,rats, rabbits, and dogs;
・The protein binding of metabolites M1 and M2 in human plasma was 98% and 95%, respectively, and was comparable across species. The extent of binding for both metabolites was constant over a wide range of concentrations (0.5 to 25 μg/mL).

Toxicology
MDV3100 has been evaluated in nonclinical toxicity studies from single doses up to 26 weeks of daily dosing, in mice, rats, dogs, and cynomolgus monkeys. Rats and dogs were the species chosen for the majority of the toxicity studies. The key findings from the MDV3100 toxicity studies were as follows:
・MDV3100 was well tolerated in rats at doses up to 100 mg/kg/day for 26 weeks and in dogs at doses up to 20 mg/kg/day for 13 weeks.
・MDV3100 was well tolerated in cynomolgus monkeys at the highest dose tested, 100 mg/kg as a single oral dose.
・Treatment-related mortality occurred in mice given ≥ 400 mg/kg as a single dose or ≥ 100 mg/kg/day for 7 days. In repeat-dose studies in rats, mortality was attributed to gavage error or accidental aspiration of the Labrasol formulation; however, no treatment-related mortality was observed at doses up to 200 mg/kg/day. In dogs, mortality at 100/60 mg/kg/day was attributed to accidental aspiration of Labrasol, but in some cases was of uncertain relationship to MDV3100.
・The main findings in repeat-dose oral studies in rats and dogs were MDV3100-related macroscopic findings, microscopic findings, and organ weight changes in reproductive and hormone-sensitive tissues. All of these tissues changes were consistent with the pharmacological activity of MDV3100. The organs affected, depending on the species or duration of dosing, were prostate glands, seminal vesicles, testes, and/or epididymides, mammary glands, pituitary glands and adrenal glands. Full or partial reversibility was noted after treatment-free periods ranging from 4 to 21 weeks.
・Clinical signs such as salivation (rats and dogs), vomiting, fecal changes (dogs and monkeys), and audible respiration (rats and mice) were attributed to the vehicle Labrasol.
・Central nervous system effects, including decreased motor activity, ataxia, clonic convulsions, and/or tremors, occurred in dose range finding toxicokinetic studies in mice

given ≥ 400 mg/kg as a single dose or 200 mg/kg/day for 7 days. One instance each of convulsions was noted in 1 dog and 1 rat in the good laboratory practice (GLP) toxicity studies.
・There were no treatment-related adverse clinical pathology findings. Mild changes, possibly attributable to the pharmacological activity of MDV3100 were noted, including increases in glucose and cholesterol, and decreases in hematology parameters (red blood cell counts, hemoglobin, and hematocrit).
・Toxicokinetic evaluations in rats and dogs showed that systemic exposure to MDV3100 generally increased with increasing dose size, but the increases were less than dose proportional. With daily oral administration, the mean accumulation index was approximately 1 to 3 in rats and 1 to 4 in dogs. The magnitude of accumulation did not appear to increase with the dose.
・The 2 major human metabolites M1 and M2 were detected in all toxicology species.
・MDV3100 was non-mutagenic in bacteria, non-clastogenic in mammalian cells, and non-genotoxic in vivo in mice.
・MDV3100 did not induce phototoxicity in cultured mammalian cells.
Consistent with the expected pharmacology of MDV3100 to inhibit the AR signaling pathway, the most salient effects of MDV3100 in rats and dogs were on male sex organs. Reductions in prostate, epididymis, and/or seminal vesicle weight were observed, and these were associated with corresponding histopathological findings of prostatic and seminal vesicle secretory depletion and/or atrophy, and with epididymal atrophy in dogs only. Hypospermatogenesis and degeneration of seminiferous tubules in testes were observed in dogs, but not in rats. Females were included in each toxicity study, except the 13-week toxicity study in dogs.

Specifically in regards to effects in females, enlarged uteri were noted in 4- and 26-week repeat-dose studies in rats only, and were considered related to MDV3100. Enlargement of the uterine lumen, observed microscopically, correlated with this finding and was reversible. Mammary glands of both male and female rats were affected histologically by MDV3100 in the 26-week study, though with qualitatively different results. Mild glandular/lumen dilationand mild lobular hyperplasia were observed in females, whereas mammary gland atrophy was observed in males. The mammary gland changes persisted after an 8-week recovery period in male and female rats. Mammary gland and uterine changes noted in female rats were not seen in female dogs in the 4-week dog study, the longest dosing duration studied in female dogs. Histopathological changes definitively related to MDV3100 treatment of dogs have been confined to changes in male sex organs.

Overall, MDV3100 was generally well tolerated in pivotal nonclinical studies with rats and dogs with the most prominent effects occurring in reproductive and hormone-sensitive tissues.

1.2.2 Summary of human experience with enzalutamide (MDV3100)

The pharmacokinetics (PK), tolerability, and antitumor activity of MDV3100 were first studied in a multi-center, open-label, first-in-human, dose-escalation study of MDV3100 in 140 patients with castration-resistant prostate cancer (S-3100-1-01).[10] Patients who were chemotherapy-naïve or who had previously failed docetaxel-based chemotherapy were treated with MDV3100 at doses of 30 to 600 mg/day until disease progression or intolerable side effects developed.

MDV3100 was absorbed rapidly after oral administration, with the time to maximum plasma concentration (tmax) after a single dose typically occurring at 1 hour postdose. No major deviations from dose proportionality were observed over the dose range 30 to 600 mg. Due to the long t1/2 (~ 5.8 days), it took approximately 1 month to reach steady state. With daily oral administration MDV3100 accumulation was observed at steady

state with an 8.3-fold higher exposure (steady-state area under the curve [AUC]) relative to a single dose. Based on the mean peak-to-trough ratio, the average difference between the peak (Cmax) and trough (minimum plasma concentration [Cmin]) concentrations was ≤ 25%. As a result of the low daily fluctuations, plasma profiles at steady-state resembled a constant infusion. The Cmin values in individual patients remained constant beyond Day 28 of chronic therapy, suggesting time-linear PK once steady state was achieved.

The maximum tolerated dose was determined to be 240 mg daily. MDV3100 demonstrated antitumor activity across endpoints in patients both with and without previous exposure to chemotherapy. The antitumor activity endpoints included prostate-specific antigen (PSA) reduction from baseline, median time to PSA progression, responses on imaging, and circulating tumor cell conversion from unfavorable to favorable counts. Three potential MDV3100-associated toxicities were identified in this study: fatigue, rash, and seizure.Three seizures (2 witnessed, 1 unwitnessed) occurred in this study at doses of 360, 480, and600 mg/day, and were reported between 26 and 48 days after initiation of MDV3100. After review of all data available from the S-3100-1-01 study, the optimal dose of MDV3100 for evaluation in Phase 3 clinical trials was determined to be 160 mg/day. A Phase 3, randomized, double-blind, placebo-controlled efficacy and safety study of oral MDV3100 (160 mg daily) in patients with progressive castration-resistant prostate cancer previously treated with docetaxel-based chemotherapy (CRPC2, also known as AFFIRM) was conducted in 1199 men, 800 of whom received treatment with MDV3100.[11]

A formal interim analysis of overall survival was performed at 520 events (80% of the 650 targeted number of events for final analysis) and demonstrated a statistically-significant increase in the duration of survival among patients treated with MDV3100 compared with patients treated with placebo (hazard ratio = 0.631 [95% CI: 0.529, 0.752], $p < 0.0001$). Median survival was 18.4 months in the MDV3100 arm and 13.6 months in the placebo arm ($\Delta = 4.8$ months). The survival benefit was seen in all pre-specified patient subgroups defined by age, geographic region, Eastern Cooperative Oncology Group (ECOG) performance status, pain score, Gleason score, number of prior chemotherapy regimens, type of disease progression at study entry, baseline level of PSA, baseline level of hemoglobin, and baseline level of lactate dehydrogenase (LDH). There were also statistically-significant increases in time to PSA progression; radiographic progression-free survival (assessed by computed tomography [CT] or magnetic resonance imaging [MRI] and by bone scan); time to first skeletal-related event; PSA response, and overall objective soft tissue radiographic response among patients treated with MDV3100 compared to placebo. In addition, statistically-significant differences favoring MDV3100 over placebo in pain palliation and pain progression rate at Week 13 were also observed.[11]

Of the MDV3100-treated patients, 98.1% reported at least 1 treatment-emergent adverse event as compared to 97.7% of placebo patients as of the data cutoff date of 25 September 2011. Serious adverse events were reported in 33.5% of MDV3100-treated patients as compared with 38.6% of placebo-treated patients. Of the MDV3100-treated patients, 7.6% discontinued study drug due to adverse events as compared with 9.8% of placebo patients.

Adverse events reported by those treated with MDV3100 with an incidence at least 2% greater than that among those who received placebo included fatigue, diarrhea, hot flush, musculoskeletal pain, headache, insomnia, anxiety, hypertension, nasopharyngitis, pollakiuria, fall, pruritus, dry skin, and musculoskeletal stiffness. Serious adverse events reported with an incidence of at least 0.5% and more frequently by MDV3100-treated patients compared to placebo patients were spinal cord compression, general physical health deterioration, hematuria, pneumonia, bone pain, metastatic pain, pathologic

fracture, urinary tract obstruction, cauda equina syndrome, pain, metastases to central nervous system, and urosepsis. The most common adverse events leading to treatment discontinuation among those receiving MDV3100 were fatigue, dysphagia, vomiting, nausea, and cerebrovascular accident, and were reported with incidence between 0.4% to 0.6%.

As an inhibitor of the GABA-gated chloride channel, MDV3100 has the potential to cause seizures.5 A dose-dependent relationship between MDV3100 exposure and seizure incidence was seen in both a nonclinical mouse study and the Phase 1 S-3100-1-01 study in which seizures were reported at supra-clinical doses in 3 patients (all with other seizure risk factors such as use of concomitant medication that may lower seizure threshold). Seizures have also been reported at the 160 mg dose; however, there were confounding factors that may have contributed to the occurrence of seizures in the majority of these cases. In the Phase 3 CRPC2 study, seizures were reported in 5 (0.6%) patients treated with MDV3100 (160 mg daily) and no placebo patients as of the data cutoff date of 25 September 2011. Two of these patients had brain metastases, 1 had inadvertently received an intravenous lidocaine overdose, 1 had recently initiated haloperidol in the context of heavy alcohol use with brain atrophy, and 1 had cortical atrophy with microvascular disease. These observations suggest that MDV3100 may lower the seizure threshold at the clinical dose of 160 mg daily. Overall, MDV3100 (160 mg daily) was well-tolerated in the CRPC2 study.

1.3 Other Agents

Synthetic peptide analogs of gonadotropin releasing hormone (GnRH) are FDA approved for palliative treatment of advanced prostate cancer. In addition, there is category 1 evidence to support the use of GnRH agonists in combination with an antiandrogen and radiation therapy for the treatment of patients with high-risk localized or locally advanced prostate cancer (NCCN Guidelines Version 1.2013). GnRH agonist treatment is a form of androgen deprivation therapy (ADT). In the current study, subjects will receive treatment with an approved GnRH agonist, leuprorelin acetate (administered as an intramuscular). Planned treatment duration will be for 2 - 3 years, at the discretion of the treating physician and the patient. Adverse effects associated with GnRH agonist treatment include diminished libido, hot flashes, hot flushes, gynecomastia, osteoporosis, increased incidence of fracture, obesity, alterations in serum lipid levels, insulin resistance, increased risk for development of diabetes mellitus, and increased risk for cardiovascular disease (NCCN Guidelines Version 1.2013).

1.4 Rationale

Prostate cancer is the leading cause of non-skin cancer in the United States, with an estimated 241,740 new cases diagnosed in 2012.[12] It is the number two cause of prostate cancer mortality among men in the US, accounting for 28,170 deaths.[12] The majority of these deaths are attributed to metastatic prostate cancer that is resistant to castration. Castration, defined as a serum testosterone level < 50 ng/dL, has been the mainstay of intervention for metastatic prostate cancer for decades. Castrate-levels of testosterone are achieved surgically through bilateral orchiectomy, or alternatively through treatment with administration of GnRH agonists or antagonists. Castrate-resistant prostate cancer (CRPC) often continues to rely on signaling through the androgen receptor that can be maintained even at very low (castrate) levels of circulating androgens. The androgen receptor signaling inhibitor enzalutamide has demonstrated activity against prostate cancer cells that overexpress the androgen receptor and are resistant to earlier generation anti-androgens such as bicalutamide.[13,14] Enzalutamide is well-tolerated and is FDA-approved for use in combination with castration therapy for the

treatment of patients with metastatic CRPC who have experienced disease progression despite docetaxel chemotherapy.[11]

Several predictors enable us to identify patients with localized prostate cancer who are at highest risk to develop metastatic CRPC. These include serum prostate specific antigen (PSA), Gleason score, and clinical tumor (T) stage on physical examination. Patients with high-risk localized, or locally advanced (those with radiographic or biopsy-demonstrated evidence of pelvic lymph node involvement) prostate cancer are routinely treated with a combination of GnRH agonist, first-generation anti-androgen, and EBRT. Androgen deprivation therapy (ADT) combined with radiation therapy has demonstrated superior survival outcomes when compared to either ADT or EBRT alone.[1-9] Standard of care treatment of men with high-risk localized or locally-advanced prostate cancer who receive external beam radiation therapy is 2-3 years of androgen deprivation therapy with a GnRH agonist, with concurrent treatment with a first-generation anti-androgen for the initial 4-6 months. EORTC 22961 demonstrated an improvement in 5 year overall survival for men with locally advanced prostate cancer treated with EBRT plus 6 months of GnRH agonist therapy plus a first-generation anti-androgen, followed by 30 months of GnRH agonist treatment alone, compared to this treatment without the additional 30 months of GnRH agonist therapy.[2] However, since many of these high-risk patients will go on to develop metastatic prostate cancer, there remains a significant need to improve upon the current treatment. No trial to date has tested the combination of enzalutamide with GnRH agonist therapy and external beam radiation therapy (EBRT) for patients with high-risk localized or locally-advanced prostate cancer. We hypothesize that this combination will be safe and well-tolerated. Once the safety profile has been established, we hypothesize that this combination may provide superior androgen signaling suppression and prostate cancer cell cytotoxicity compared to a first-generation anti-androgen plus GnRH agonist and improve clinical outcomes.

This pilot study will assess the safety and tolerability of combining enzalutamide with GnRH agonist treatment and EBRT. We hypothesize that administration of enzalutamide plus a GnRH agonist for 6 months in combination with EBRT, followed by treatment with a GnRH agonist alone for 18 - 30 months, will be a safe and well-tolerated treatment for patients with high-risk localized or locally advanced prostate cancer. We will perform correlative imaging and tumor tissue biopsy analysis to assess the radiographic response to enzalutamide plus GnRH therapy as well as intratumoral androgen signaling.

The primary endpoint is to determine the safety and tolerability of combining enzlutamide plus a GnRH agonist with EBRT up to 12 months following initiation of treatment with enzalutamide plus GnRH agonist. Primary endpoint measures are frequency of AEs with monitoring for

1. Expected toxicities specific to the combination of enzalutamide plus GnRH agonist based on the clinical experience with this FDA-approved combination for the treatment of metastatic castration-resistant prostate cancer as annotated in section 1.2.2
2. Expected radiation-related toxicities as annotated in section 4.4.1.8
3. Unexpected severe AEs due to combination of enzalutamide plus GnRH agonist with EBRT.

Secondary endpoints will include

1. Molecular markers of androgen signaling and cell proliferation in prostate biopsy samples
    a. Inhibition of androgen-regulated gene expression (potential targets to include NDRG1, FKBP5, TMPRSS2, and PSA) by qRT-PCR
    b. Inhibition of androgen receptor (AR) nuclear localization and prostate cancer cell proliferation measured by immunohistochemistry (IHC)
    c. Androgen metabolism in prostate biopsy samples

2. Serum PSA and testosterone following combination therapy with enzalutamide plus GnRH agonist therapy prior to initiation of radiation therapy
3. Changes in prostate tumor volume and activity following 2 months of combined enzalutamide plus GnRH therapy compared to baseline as measured by multi-parametric magnetic resonance imaging (MRI)

These results will lay the groundwork for subsequent randomized trials to compare this treatment combination with the current standard of care in patients with high-risk localized and locally-advanced prostate cancer.

1.5 Correlative Studies

In pre-clinical studies, enzalutamide was shown to inhibit androgen receptor signaling through multiple mechanisms.[13,14] Enzalutamide inhibits androgen-induced receptor activation (binding of androgens to ARs in the cytosol), inhibits nuclear translocation of activated ARs, and inhibits the association of the activated AR with chromatin, even in the setting of AR overexpression and in prostate cancer cells resistant to anti-androgens such as bicalutamide. However, inhibition of AR signaling by enzalutamide has not been reported directly in primary patient tumor samples. We hypothesize that enzalutamide will impair AR nuclear localization and expression of AR-regulated genes in human tumors when administered in combination with GnRH agonist treatment. We hypothesize that this treatment combination will also result in a measurable anti-tumor response by muli-parametric magnetic resonance imaging (MRI).

To test these hypotheses, we will obtain prostate cancer tumor biopsies at the time of study enrollment and following two months of treatment with enzalutamide plus GnRH agonist. MRI of the pelvis, protocolled to assess the prostate, will be performed prior to the initial biopsy and again prior to the subsequent biopsies obtained following two months of treatment.

2.0 STUDY OBJECTIVES

2.1 Primary Objectives

2.1.1 To determine the safety and tolerability of combining enzalutamide 160 mg by mouth daily with a GnRH and EBRT. Enzalutamide and GnRH agonist will be administered in combination for 6 months, beginning two months prior to the start of EBRT. Enzalutamide and GnRH agonist treatment will continue for the duration of EBRT and for approximately two months after completion of EBRT, for a total of 6 months of combined enzalutamide plus GnRH treatment. Patients will subsequently receive an additional 18 - 30 months of GnRH agonist therapy for a total of two - three years of GnRH agonist treatment. Safety and tolerability will be assessed by frequency and severity of adverse events with monthly monitoring for 12 months following initiation of enzalutamide plus GnRH therapy.

Secondary Objectives

2.1.2 To determine whether enzalutamide combined with GnRH agonist treatment inhibits intra-tumoral androgen-regulated gene expression (potential targets to include NDRG1, FKBP5, TMPRSS2, and PSA) as measured by qRT-PCR

2.1.3 To determine whether enzalutamide combined with GnRH agonist treatment impairs androgen receptor nuclear localization and cancer cell proliferation in targeted prostate cancer biopsy samples.

2.1.4 To determine the impact of enzalutamide combined with GnRH agonist on androgen metabolism in prostate biopsy samples.

2.1.5 To determine the impact of enzalutamide plus GnRH agonist therapy on serum prostate specific antigen (PSA) and testosterone following two months of treatment.

2.1.6 To describe any preliminary evidence of anti-tumor activity by assessment of objective response as determined by multiparametric MRI in patients with high-risk localized or locally-advanced prostate cancer.

2.2 Exploratory Objectives

Exploratory objectives are those defined above as secondary objectives 2.1.2 – 2.1.4.

2.3 Endpoints

2.1.1 Safety and tolerability will be assessed based on the rate of drug-related grade 1-5 adverse events experienced within the first 12 months of study treatment. These will be assessed via NCI's CTCAE v4.0 toxicity criteria.

2.1.2 Molecular markers of androgen-regulated gene expression in tumor biopsy samples will be assessed by qRT-PCR at the time of study initiation and following two months of combined treatment with enzalutamide plus GnRH agonist.

2.1.3 Androgen receptor nuclear localization and cancer cell proliferation (using Ki-67) will be analyzed by immunohistochemical analysis of tumor biopsy samples at the time of study initiation and following two months of combined treatment with enzalutamide plus GnRH agonist.

2.1.4 Enzymes of androgen metabolism will be assessed from prostate tumor biopsy specimens by qRT-PCR

2.1.5 Serum levels of prostate specific antigen (PSA) and testosterone will be measured at study initiation and following two months of treatment with enzalutamide combined with GnRH agonist.

2.1.6 Changes in prostate tumor volume and tumor activity will be measured by magnetic resonance imaging (MRI) at the time of study initiation prior to the initial study biopsy and again following two months of combined enzalutamide plus GnRH agonist treatment just prior to the second study biopsy to be performed at the time of fiducial marker placement.

3.0 Subject ELIGIBILITY

Eligibility waivers are not permitted. Subjects must meet all of the inclusion and exclusion criteria to be registered to the study. Study treatment may not begin until a subject is registered.

3.1 Inclusion Criteria

3.1.1 Histologically proven adenocarcinoma of the prostate obtained within 6 months of screening. Patients in whom a diagnosis of high-risk localized or locally-advanced prostatic adenocarcinoma is suspected based on a serum PSA > 20

ng/mL or clinical T3 disease by digital rectal examination, but who have not yet undergone diagnostic prostate biopsy, will be eligible for screening and initial MRI and targeted prostate cancer biopsies which will be obtained at the same time as diagnostic biopsies. Those patients in whom the diagnostic biopsies confirm prostatic adenocarcinoma will be permitted to continue with study treatment if they meet all additional eligibility criteria.

3.1.2 Age ≥ 18 years.

3.1.3 Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2

3.1.4 Adequate organ and marrow function as defined below:

- leukocytes	≥ 3,000/mcL
- absolute neutrophil count	≥ 1,500/mcL
- platelets	≥ 100,000/mcl
- hemoglobin	≥ 10 g/dL
- total bilirubin	≤ 1.5 X institutional upper limit of normal (except for patients with documented Gilbert's disease)
- AST(SGOT)/ALT(SPGT)	≤ 2.5 X institutional upper limit of normal
- creatinine	≤ 1.5 X institutional upper limit of normal or estimated glomerular filtration rate < 45 mL/min/1.73m2, given the risk of nephrogenic systemic fibrosis when using gadolinium-based intravenous contrast agents in these subjects.
- serum testosterone	≥ 100 ng/dL

3.1.5 Men who are sexually active with female partners of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation, and for *90* days following completion of therapy. Should a woman become pregnant or suspect she is pregnant following intercourse with a study participant during his participation in this study, she should inform her treating physician immediately, and the study principal investigator should be informed immediately.

3.1.6.1 A female of child-bearing potential is any woman (regardless of sexual orientation, having undergone a tubal ligation, or remaining celibate by choice) who meets the following criteria:

- Has not undergone a hysterectomy or bilateral oophorectomy; or
- Has not been naturally postmenopausal for at least 12 consecutive months (i.e., has had menses at any time in the preceding 12 consecutive months).

3.1.6 Patients must be candidates for long-term androgen deprivation in combination with EBRT for the treatment of high-risk or locally-advanced prostate cancer by the following criteria:

- High risk disease: T3a or Gleason 8-10 or serum PSA > 20 ng/mL
- Gleason 7 also allowed if > 50% of cores positive for cancer or PSA velocity > 2 ng/mL/year in preceding 12 months
- Locally advanced (very high risk) disease: T3b-T4

3.1.7 Patients may have radiographic evidence of metastasis in regional lymph nodes (N1 disease as defined by the National Comprehensive Cancer Network Prostate Cancer Guideline Version 3.2012) at the discretion of the treating physicians, if regional lymph nodes can be included in the planned radiation field.

3.1.8 Participants in the study must permit targeted prostate biopsy prior to initiation of study treatment and at the time of fiducial marker placement

3.1.9 Able and willing to provide written authorization for use and release of health and research study information

3.1.10 Ability to understand and the willingness to sign a written informed consent.

3.2 Exclusion Criteria

3.2.1 Prior treatment with the following agents known to have endocrine effects on prostate cancer: GnRH agonist, GnRH antagonist, anti-androgen (bicalutamide, nilutamide, flutamide), ketoconazole, diethylstilbestrol, estrogen, abiraterone acetate. Concurrent use of 5α-reductase inhibitors finasteride or dutasteride is permitted for patients who have been already receiving either of these treatments for at least 1 month at the time of study enrollment. Baseline PSA must have been obtained in such patients after at least 1 month on 5α-reductase treatment. Initiation of treatment with 5α-reductase inhibitors is not permitted within the first 12 months of study participation.

3.2.2 Concomitant treatment with agents thought to have endocrine effects on prostate cancer: PC-SPES, saw palmetto

3.2.3 Treatment with corticosteroids within 4 weeks prior to enrollment.

3.2.4 Treatment with androgens within 6 months prior to study enrollment.

3.2.5 Subjects may not be receiving any other investigational agents. Concurrent enrollment in another clinical investigational drug or device study is prohibited.

3.2.6 Prostate cancer metastases to the bones, viscera, or non-regional lymph nodes (lymph nodes other than pelvic lymph nodes within the radiation treatment field)

3.2.7 Serum PSA > 160 ng/dL

3.2.8 History of malignancy (other than non-melanoma skin cancer) within 5 years of enrollment

3.2.9 Patients with histologic evidence of small cell carcinoma of the prostate will not be eligible.

3.2.10 Treatment for malignancy with anticancer therapy, including cytotoxic agents, hormonal agents, or immunotherapy, within 5 years of enrollment

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

3.2.12 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure (> NYHA class II hear failure), unstable angina pectoris, cardiac arrhythmia, chronic active hepatitis, acute hepatitis, uncontrolled diabetes mellitus, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.13 Patients with any of the following within 6 months of enrollment: deep vein thrombosis, pulmonary embolus, myocardial infarction, cerebrovascular accident, unexplained loss of consciousness.

3.2.14 Patients with any history of seizure or seizure disorder.

3.2.15 Patients with medical conditions which, in the opinion of the investigators, would pose undue risk to the patient

3.2.16 Subjects unwilling to use contraceptives if they have sexual intercourse with a female partner of child-bearing potential while receiving treatment on this study.

3.2.17 Standard contraindications to MRI: For example, MRI non-compatile cardiac pacemakers, intracranial clips, foreign metal objects in the body and others as defined in the UT Southwestern Institutional MRI Safety Policy)

3.2.18 The following medications are prohibited during the study:
- Substrates of CYP2B6, CYP2C8, CYP2C9, and CYP2C19 with a narrow therapeutic index, including paclitaxel, phenytoin, warfarin, omeprazole
- Substrates of CYP3A4 with a narrow therapeutic index, including alfentanil, cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus, tacrolimus
- Strong CYP2C8 inhibitors, including gemfibrozil
- Strong CYP3A4/5 inhibitors, including clarithromycin, itraconazole, ketoconazole

4.0 TREATMENT PLAN

4.1 Treatment Dosage and Administration

4.1.1 We propose a single-institution, single-arm study of neo-adjuvant, concomitant, and adjuvant enzalutamide plus GnRH agonist with external beam radiation therapy for the treatment of patients with high-risk and locally-advanced prostate cancer.

4.1.2 Eligible study participants will receive treatment with enzalutamide 160 mg daily for 6 months in combination with GnRH agonist treatment. Following the initial two months of treatment with enzalutamide and GnRH agonist, radiation therapy will be administered as detailed below. GnRH agonist treatment will be continued for a total treatment duration of 24 - 36 months at the discretion of the treating physician in concert with the patient. Treatment will be administered on an outpatient basis.

Table 4.1 **Agent**	**Dose**	**Route**	**Schedule**
Enzalutamide	160 mg daily (four 40 mg capsules)	p.o.	Daily for 6 months
Leuprolide acetate	22.5 mg every 3 months or 45 mg every 6 months	IM	Every 3 or 6 months (depending on dosage form used) for a total treatment duration of 24-36 months

A study medication diary will be provided to each subject in which daily oral self-administration of enzalutamide will be documented. If a dose is missed, enzalutamide should be resumed the next day at the established daily dose of 160 mg. A vomited dose should not be replaced.

4.2 Toxicities and Dosing Delays/Dose Modifications

Any subject who receives treatment on this protocol will be evaluable for toxicity. Each patient will be assessed for the development of toxicity according to the Time and Events table (Table 5.4). Toxicity will be assessed according to the NCI Common Toxicity Criteria for Adverse Events (CTCAE), version 4.0. Dose adjustments should be made according to the system showing the greatest degree of toxicity.

Enzalutamide (Xtandi, MDV3100) has been shown to be well-tolerated when used in combination with androgen deprivation (in the form of GnRH agonists or castration by orchiectomy) in completed phase I, II, and III clinical trials.

Table 4.2.1 Hematological Toxicity Dose Reductions for Enzalutamide

ANC	Platelets	Action
≥ 1,500/μL	100,000/μL	None.
1000-1499/μL	75,000-99,000/μL	-*1st Occurrence:*Hold current dose until ANC ≥ 1,500/μL and platelets ≥ 100,000/μL. Do not replace missed doses. Restart next treatment at 120 mg dose. -*2nd Occurrence*:Hold current dose until ANC ≥ 1,500/μL and platelets ≥ 100,000/μL. Do not replace missed doses. Restart next treatment at 80 mg dose. -*3rd Occurrence:* Discontinue protocol therapy.
500-999/μL	50,000-74,000/μL	-*1st Occurrence*:Hold current dose until ANC ≥ 1,500/μL and platelets ≥ 100,000/μL. Do not replace missed doses. Restart next treatment at 120 mg dose. -*2nd Occurrence*: Discontinue protocol therapy.
<500/μL	<50,000/μL	-*1st Occurrence*: Discontinue protocol therapy.

Table 4.2.2. Non-hematological Toxicity Dose Reductions

NCI CTC Grade	Enzalutamide
0-2	No change from original starting dose **(with the exception of seizure)**
3-4	Hold until resolved to ≤Grade 2, then reduce **to 120 mg dose**
Second episode of grade 3 or 4 toxicity	Hold until resolved to ≤Grade 2, then reduce **to 80 mg dose**
Third episode of grade 3 or 4 toxicity	Remove subject from trial

In the randomized clinical trial of patients with docetaxel-refractory metastatic CRPC, 7 of 800 patients (0.9%) treated with enzalutamide 160 mg daily experienced a seizure. No seizures occurred in patients treated with placebo. Patients experiencing seizures were permanently discontinued from therapy and all seizures resolved. There is no clinical trial experience re-administering enzalutamide to patients who experienced seizures. Therefore, should a subject experience any seizure, enzalutamide will be discontinued and the subject removed from the trial.

4.3 Concomitant Medications/Treatments

In vitro studies show that enzalutamide and/or metabolite M2 are potential inhibitors of CYP2C8 and CYP2C19 with lesser potential for inhibitory effects on CYP2B6 and CYP2C9. Enzalutamide is a moderate CYP2C9 and CYP2C19 inducer in humans. For this study, substrates of CYP2B6, CYP2C8, CYP2C9, and CYP2C19 that have a narrow therapeutic index (e.g., paclitaxel, phenytoin, warfarin, omeprazole) are prohibited.

In vitro studies show that enzalutamide is an inducer of CYP3A4. Induction of CYP3A occurs via activation of the nuclear pregnane X receptor (PXR), which is expected to result in co-induction of CYP2C. Co-administration of enzalutamide with CYP3A or CYP2C substrates may reduce oral bioavailability and/or accelerate elimination of these substrates. Enzalutamide is a strong CYP3A4 inducer in humans. For this study, 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) is prohibited.

In vitro studies show that enzalutamide is metabolized by CYP2C8 and CYP3A4/5. Strong inhibitors or inducers of these enzymes may affect enzalutamide exposures. Co-administration of a strong CYP2C8 inhibitor (gemfibrozil) increased the composite area under the plasma concentration-time curve (AUC) of enzalutamide plus N-desmethyl enzalutamide in healthy volunteers. For this study, co-administration of enzalutamide and strong CYP2C8 inhibitors (e.g., gemfibrozil) is prohibited. Co-administration of a strong CYP3A4 inhibitor (itraconazole) increased the composite AUC of enzalutamide plus N-desmethyl enzalutamide by 1.3 fold in healthy volunteers. For this study, strong inhibitors of CYP3A4/5 (e.g., clarithromycin, itraconazole, ketoconazole) are prohibited during enzalutamide treatment.

Use caution when co-administering strong inducers of CYP2C8 (e.g., rifampin) or CYP3A4/5 (e.g., carbamazepine, phenytoin, rifampin, St. John's wort) during enzalutamide treatment, as enzalutamide concentrations may decrease.

In vitro studies show that enzalutamide and metabolite M2 are potential inhibitors of the efflux transporter P-gp. Co-administration of enzalutamide with P-gp substrates may increase the plasma concentrations of the P-gp substrate. Use caution when co-administering sensitive P-gp substrates (e.g., colchicine, dabigatran etexilate, digoxin) during enzalutamide treatment.

Food has no clinically significant effect on the extent of absorption. Enzalutamide may be taken with or without food.

Osteoporosis is a potential adverse effect of GnRH agonist therapy. As per the NCCN Prostate Cancer Guidelines Version 1.2013, screening and treatment for osteoporosis are recommended according to guidelines for the general population provided by the National Osteoporosis Foundation (www.nof.org). It is advised that subjects enrolled in this study be treated for osteoporosis at the discretion of the investigator according to NOF guidelines. These recommendations include treatment with supplemental calcium (1200 mg daily) and vitamin D3 (800-1000 IU daily) for all men > 50 years of age and

additional treatment for men with a 10 year probability of hip fracture ≥ 3% or 10 year probability of a major osteoporosis-related fracture ≥ 20% as assessed using the FRAX algorithm established by the World Health Organization. Using the FRAX algorithm, GnRH agonist treatment should be considered a secondary osteoporosis risk factor. Osteoporosis reatment options permitted in conjunction with this study for appropriate patients include denosumab 60 mg SQ every 6 months x 6 doses, zoledronic acid 5 mg iv annually, or alendronate 70 mg PO weekly.

Medications known to lower the seizure threshold include but are not limited to:

- Aminophylline/theophylline;
- Atypical antipsychotics (e.g., clozapine, olanzapine, risperidone, ziprasidone);
- Bupropion;
- Lithium;
- Pethidine (meperidine);
- Phenothiazine antipsychotics (e.g., chlorpromazine, mesoridazine, thioridazine);
- Tricyclic and tetracylic antidepressents (e.g., amitriptyline, desipramine, doxepin, imipramine, maprotiline, mirtazapine).

4.4 Other Modalities or Procedures

4.4.1 RADIATION THERAPY

Radiotherapy should begin within 8-10 weeks(+/- 7 days) after the date of the GnRH and enzalutamide administration.injection. Radiotherapy will be administered as standard of care treatment.

4.4.1.1. ***Plan 1***: Whole pelvis including prostate and seminal vesicles
Acceptable Treatment Modalities:
3D-CRT or IMRT. For patients with N1 disease, only IMRT plan will be allowed.

Prescription Dose (See Table 4.4.1.1):
45 Gy to cover 98% (+/- 3%) of PTV
・Minimum dose within PTV – 95% (+/- 5%) of prescribed dose and for a volume that is 0.03 cc
・Maximum dose within the PTV – 107% (+/- 3%) of prescribed dose and for a volume that is 0.03 cc

In patients with N1 disease, PTV-N will be defined:
Prescription dose (See table 4.4.1.2)
55 Gy to cover 95 % (+/- 5%) of PTV-N

- Minimum dose within PTV – 93% (+/- 3%) of prescribed dose and for a volume that is 0.03 cc
- Maximum dose within the PTV – 105% (+/- 5%) of prescribed dose and for a volume that is 0.03 cc

Table 4.4.1.1: Radiation Therapy Dose Objectives for Plan 1 – Pelvic and Prostate Radiation

PTV dose (encompassing 98% PTV (+/- 3%))	Minimum PTV dose for a point with a volume of .03cc	Maximum PTV dose for a point with a volume of .03cc	Maximum PTV dose to a volume of .03 cc of PTV (variation acceptable)	Maximum PTV dose to a volume of .03 cc of PTV (Deviation unacceptable
45 Gy	42.8 Gy	48.2 Gy	> 48.2-49.5 Gy	>49.5 Gy

*Maximum dose must not be within an organ at risk (OAR) such as the rectum, bladder.

Table 4.4.1.2: Radiation Therapy Dose Objectives for Plan 1 – PTV-N

PTV-N dose (encompassing 95% PTV (+/- 3%))	Minimum PTV-N dose for a point with a volume of .03cc	Maximum PTV-N dose for a point with a volume of .03cc	Maximum PTV-N dose to a volume of .03 cc of PTV (variation acceptable)	Maximum PTV-N dose to a volume of .03 cc of PTV (Deviation unacceptable
55 Gy	50.06 Gy*	60.05 Gy	> 57.75-60.05 Gy	>60.05 Gy

***if PTV-N is adjacent to small bowel, minimum acceptable PTV-N dose will be dose that can be achieved while meeting absolute small bowel dose constraints.**

4.4.1.2. Plan *2:* Reduce volume cone down boost plan to the prostate and (proximal) seminal vesicles
Acceptable Treatment Modalities:
IMRT or 3DCRT

Table 4.4.2 : Radiation Therapy Dose Objectives for Plan 2 – Prostate and Seminal Vesicle Boost

PTV dose (encompassing 98% PTV (+/- 3%))	Minimum PTV dose for a point with a volume of .03cc	Maximum PTV dose for a point with a volume of .03cc	Maximum PTV dose to a volume of .03 cc of PTV (variation acceptable)	Maximum PTV dose to a volume of .03 cc of PTV (Deviation unacceptable
34.2 Gy	32.49 Gy	37.62 Gy	> 36.6-37.6 Gy	>37.6 Gy

Maximum dose must not be within an OAR such as rectum, or bladder.

4.4.1.3 Technical Factors
Either 3DCRT or IMRT may be used for treatment. For 3DCRT treating the whole pelvis (WPRT), a minimum of 4-fields should be used and a 4 field plan is recommended. For IMRT, no specific field arrangement is required. RT will be delivered with megavoltage equipment at energies ≥ 6 MV. Typically, except for VMAT techniques, 5 to 9 gantry angles are employed for the IMRT treatments. When using IMRT, preference will be given to using photon energies <=10MV, but higher energies will be permitted if necessary for production of a more optimal plan due to anatomic constraints.

4.4.1.4 EBRT Localization, Simulation, and Immobilization
Simulation will be CT-based in all cases. A urethrogram or MRI is recommended, but not required, to be used for target delineation. Rectal contrast is discouraged because it may distend the rectum and artificially displace the prostate in the anterior direction. Intravenous contrast is permitted but not required to assist in identifying the pelvic vessels. Patients will be positioned supine or prone on a flat tabletop with a customized thermoplastic immobilization cast or a molded foam cradle or similar immobilization for stabilization and setup reproducibility. The degree of bladder fullness should be made to duplicate the degree of fullness anticipated for daily treatment, i.e., if the patient is instructed to maintain a full bladder for treatment, he should be simulated as such (especially for cases in which image guidance is not implemented). The rectum should be kept as empty as possible; recommendation is for an enema 1-2 hours prior to simulation. CT images should be acquired at a slice thickness of ≤3 mm from L3/L4 vertebral

body to the perineum inferiorly. If Calypso beacons are used, slice thickness of 1.5-2 mm will be recommended. Target volumes and normal critical structures (as defined in section 4.4.1.5.3) will be defined in the slices in which they are visualized. The 3DCRT cases must utilize "beam's eye view" representations to define final beam aperture.

4.4.1.5 Treatment Planning/Target Volumes

4.4.1.5.1 Patients must have a composite treatment plan generated at the beginning of Plan 1 so that the final EBRT dose to critical structures is evaluated before any dose delivery has begun. Dose for initial treatment (CTV1/PTV1) will be 45.0 Gy at 1.8 Gy per fraction. If patient has N1 disease, CTV-N/PTV-N will be defined, and will received 55 Gy at 2.2 Gy per fraction during plan 1of the treatment course using simultaneous integrated boost technique (SIB). All patients with N1 disease will be treated with IMRT, and 3DCRT will not be permitted. Once this portion is completed, a cone down boost to the prostate and (proximal) seminal vesicles will be delivered by either IMRT or 3DCRT. Brachytherapy boost is not permitted on this study. For the boost, the dose to the CTV2/PTV2 will be 34.2 Gy at 1.8 Gy per fraction, for a total prostate dose of 79.2 Gy. For pelvic 3D-CRT, a 4-field technique, using opposed anterior-posterior and opposed lateral fields, is recommended. All fields should conform to the beam's-eye-view of the target. No specific field arrangement is required for IMRT, although typically 5-9 fields are used for fixed gantry treatment. Tomotherapy and VMAT/rapid arc/ smart arc IMRT technologies) also are allowed for IMRT treatment on this protocol.

4.4.1.5.2. The definition of GTV, CTV and PTV will be in accordance with the ICRU Report #50: Prescribing, Recording, and Reporting Photon Beam Therapy.

4.4.1.5.2.1 *Plan 1 Pelvis, including prostate and seminal vesicles*

Gross Target Volume (GTV1 and GTV-N)

The GTV1 is defined by the physician as all known disease as defined by the planning CT, urethrogram, MRI, and clinical information. If a urethrogram is used, the GTV will encompass a volume inferiorly 5 mm superior to the tip of the dye and no less than the entire prostate. Prostate dimensions should be defined as visualized on CT scan. If MRI is performed, fusion of planning CT with the MRI will be performed on the treatment planning system, and MRI will be utilized to help further refine and delineate the GTV.

GTV-N will be defined as all positive pelvic nodal disease > 1.5 cm in short axis diameter seen on CT scan or MRI before initiation of hormone therapy. Nodal disease will be contoured based on the pre-hormone therapy imaging studies. If necessary, a CT simulation can be performed prior to initiation of hormone therapy as a baseline study that can be used for treatment planning purposes. At the discretion of the treating physician, nodal disease >=1cm will also be considered for GTV-N, particularly if there are multiple gross nodal disease present.

Clinical Target Volume (CTV1)

The CTV1 will include the prostate and entire seminal vesicles (SV), the obturator, external iliac, proximal internal iliac and distal common iliac nodes, using the vascular structures, up to a level corresponding to the top of L5-S1. At the treatment team's discretion, coverage can be increased to include common iliac nodes up to L4/L5 level. One can refer to the pelvic nodal atlas at the RTOG Web site (Pelvic Lymph Node Volumes for Prostate Cancer Atlas; **http://www.rtog.org/CoreLab/ContouringAtlases/ProstatePelvicLymphNodes.aspx**).
The presacral nodes from L5-S1 to S3 may be included if desired depending on whether the dose constraints to the rectum are achievable (see Table 4.4.3). The CTV1 will include a 7 mm margin in 3-dimensions to the contoured iliac vessels, but not extend outside of the true pelvis, into the pelvic musculature nor into adjacent identifiable organs, such as the bladder, rectum or other bowel. Extension of the CTV into adjacent bone may be carved out. Anatomical boundaries to consider if 3DCRT is used include superiorly from L5-S1 to 0.5 cm below the tip of the urethral contrast dye inferiorly to ensure that the entire prostate gland is included (MRI can also be used to help define the apex). Lateral borders will be at least 1 cm from the pelvic brim. In the lateral fields, the external and internal iliac lymph nodes below the SI joints, and the posterior extension of the seminal vesicles should be covered. The usual posterior border is approximately S2-3, but

CT anatomy should take precedence. The inferior extent of the external iliac lymph nodes is at the top of the femoral heads. The inferior extent of the obturator lymph nodes is at the top of the symphysis pubis.

If there are grossly involved nodes seen in the pelvis, this can be treated to a higher dose using a simultaneous integrated boost technique (see section 4.4.1.1 and Table 4.4.1.2). For these patients, a GTV-N will be defined as the grossly evident node based on imaging studies (> 1.5 cm for solitary, and > 1 cm for multiple nodes at the discretion of treating physician) (see section 4.4.1.5.2.1). Imaging studies prior to hormone therapy can be used to guide in the delineation of GTV-N. Alternatively for node positive patients, a CT simulation in the treatment position can be performed prior to hormone therapy to be used in fusion with CT simulation to be performed at a later date to help define the nodal volume. CTV-N will be defined as being GTV-N + 7mm margin in 3-dimensions. As always careful consideration will be made in defining CTV to carve disease out of the bowel.

Planning Target Volume
The PTV1 margins should be a minimum of 0.5 cm and a maximum of 1.5 cm in all dimensions.

4.4.1.5.2.2 *Plan 2 Prostate and Proximal Seminal Vesicles Boost with IMRT*
Gross Target Volume (GTV 2)
See Section 4.4.1.5.2.1 above for GTV1.

Clinical Target Volume (CTV2)
The CTV2 is the GTV2 plus areas considered to contain microscopic disease, delineated by the treating physician. The CTV2 includes the GTV (prostate) plus areas at risk for microscopic disease extension plus the proximal bilateral seminal vesicles. Typically, only the proximal 1.0 cm of seminal vesicle tissue adjacent to the prostate shall be included in the clinical target volume. This 1.0 cm of seminal vesicles refers to both radial (in plane) and superior (out of plane) extent. If both prostate and seminal vesicle are visualized in the same CT slice, this seminal vesicle tissue will contribute to the 1.0 cm of tissue as seen on the CT simulation scan. For patients with clinical biopsy proven involvement of the seminal vesicles, or with imaging evidence of seminal vesicle involvement, or with clinical suspicion at the discretion of treating physician, treatment of > 1 cm of the proximal seminal vesicles (up to entire seminal vesicle), is acceptable as long as it does interfere with the ability to achieve dose constraints to the critical normal structures (see section 4.4.1.5.3).

Planning Target Volume (PTV2)
The PTV2 will provide a margin around the CTV2 to compensate for the variability of treatment set up and internal organ motion. A range of 5-10 mm around the CTV is required to define each respective PTV. Individual selection of a PTV margin should be based on the institution's level of confidence in patient set-up and the availability of image guidance. Superior and inferior margins (capping) should be 5-10 mm depending on the thickness and
spacing of the planning CT scan. Careful consideration should be made when defining the 5- 10 mm margin in 3 dimensions.

4.4.1.5.3 Normal Critical Structures and Dose Constraints
Normal critical structures to be defined on the treatment planning CT scan will include the following: bladder, rectum (from its origin at the rectosigmoid flexure superiorly or the bottom of the SI joints, whichever is more inferior to the inferior-most extent of the ischial tuberosities), sigmoid colon (to the level of superior border of PTV1), bilateral femora (to the level of ischial tuberosity), penile bulb, and skin. Any small bowel within the primary beam aperture should be defined as well. The normal tissues will be contoured and considered as solid organs. The bladder should be contoured from its base to the dome, and the rectum from the anus (at the level of the ischial tuberosities) for a length of 15 cm or to the rectosigmoid flexure. This generally is below the bottom of the sacroiliac joints. If IMRT is being used to treat the pelvic nodes, the potential bowel space (not just individual loops of bowel) where the small and large bowel may

fall should be outlined. The borders are the abdominal wall anteriorly, pelvic sidewalls laterally (excluding the pelvic lymph node regions), superiorly to one cut above the last axial CT image on which the lymph nodes are outlined and inferiorly from the level of the top of PTV1 (outlining around the sides of the bladder near the top of the bladder to encompass the bowel that may fall into these regions). See the ITC web site (http://atc.wustl.edu) to view examples of target and normal tissue contours.
The following table summarizes the dose constraints for the critical structures.

Table 4.4.3 Organs at Risk, and Dosimetric Constraints

	≤ 15%	≤ 20%	≤ 35%	≤ 50%
Rectum/Sigmoid	75 Gy	70 Gy	65 Gy	40 Gy
	10 cc < 75.6 Gy 20 cc < 70 Gy			
Bladder	80 Gy	75 Gy	70 Gy	50 Gy
	90 cc < 70 Gy 150 cc < 65 Gy			
Potential Bowel Space	0.03 cc < 50.4 Gy* 50 cc < 45 Gy*			
L and R Femur	≤ 5% receives 50 Gy			
Penile bulb	Mean dose ≤ 52.5 Gy			

While every effort should be made to deliver prescription doses to the PTV as specified while adhering to these constraints, it is recognized that certain anatomical factors may prevent this. Prescription dose reduction to a level of 77.4 Gy or 75.6 Gy is permitted if constraints cannot be met at a prescription dose of 79.2 Gy. For purposes of compliance, up to a 5% absolute increase in the volume of critical structure receiving greater than the specified dose will be considered "variation acceptable," e.g. up to 20% of the rectum may receive a dose of > 75.6 Gy without a protocol deviation. Any increase in critical structure volume greater than 5% receiving more than the specified dose will be considered a "deviation unacceptable." It is at this point that a dose reduction to 77.4 Gy or 75.6 Gy should be implemented. The prescription dose should be the maximum deliverable up to 79.2 Gy while respecting the critical normal structure constraints. Of note, the penile bulb constraint is to be regarded as a guideline, and adherence to this should not, in any way, result in a reduction of the prescription dose or compromised dose coverage of the target volume. In the event that PTV-N is in close proximity to the small bowel, absolute small bowel constraints will be prioritized over PTV-N coverage. * variation will be noted if > 50 cc to 150 cc of potential small bowel space receives ≥ 45 Gy. A secondary variation will be noted if > 150 cc receives >45 Gy. However, at the discretion of the treating physician, will consider max point dose up to 55 Gy.

4.4.1.6 Treatment Verification

4.4.1.6.1 For 3DCRT: First day port films or portal images of each field along with orthogonal isocenter verification films (or images) must be obtained. If modifications are made in field shaping or design, a port film/image of each modified field along with orthogonal isocenter verification films (or images) is required on the first day's treatment of that field. Thereafter, weekly verification films or images of orthogonal isocenter views (anterior to posterior and lateral projection) are required.

For IMRT: the intensity profiles of each beam must be independently verified and compared to the planned field intensity. Portal films/images are required for IMRT but orthogonal verification films/images are required, just as for 3DCRT. Real-time ultrasound localization and on-line cone beam CT image guidance are important complements to conventional port films or portal imaging and should be used when available. Weekly port filming/imaging is required in this study.

4.4.1.6.2 Daily on-line target localization (kV or MV imaging with fiducials, kV or MV ConeBeam CT, trans-abdominal ultrasound, or other) or off-line adaptive approaches to account for interfraction organ motion and setup variability are permitted on this study. Fiducials (gold seeds) will be placed by the UT Southwestern Urology department at least 10-14 days prior to the intiation of radiation therapy date and details for this process are outlined in 4.4.2. Calypso Beacons are permitted to be used in place of gold seed fiducial markers, if monitoring intrafractional motion is desired at the discretion of the treatment team.

4.4.1.7 Quality Assurance
4.4.1.7.1 Compliance Criteria for Cases Treated with EBRT
Cases that are treated entirely with external beam radiation therapy must meet the criteria as stated in Sections 4.4.1.1 and 4.4.1.2 (see also Tables 4.4.1.1, 4.4.1.2, and 4.4.2). Both the Plan 1 and 2 requirements must be met. In addition, the critical structure dose constraints of Section 4.4.1.5.3 and Table 4.4.3 must be met.
4.4.1.7.1.1 Acceptable dose heterogeneity for external beam treatment is summarized in Tables 5.1 and 5.2. The treating physician must carefully consider the tolerance dose/volume to each critical normal structure and unspecified tissue.
4.4.1.7.2 Radiation Quality Assurance Review
The study's radiation PI or his designees will perform an RT Quality Assurance remote review after complete data for the first 4 of the cases enrolled has been made available. The study PI or his designees will perform the next remote review after complete data for the next 3 cases have been enrolled. The final cases will be reviewed within 3 months after this study has reached the target accrual or as soon as complete data for all cases enrolled has been made available, whichever occurs first. These reviews will be ongoing and performed remotely.

4.4.1.8. Radiation Therapy Adverse Events
All patients will be seen weekly by their treating radiation oncologist while undergoing EBRT. Any observations with respect to the following symptoms/side effects will be recorded: Bowel/rectal irritation manifesting as cramping, diarrhea, urgency, proctitis, or hematochezia, urinary frequency, urgency, dysuria, hematuria, urinary tract infection, or incontinence. CTCAE 4.0 will be used to grade all toxicities.

4.4.1.8.1. Clinical discretion may be used in managing radiotherapy-related side effects. Diarrhea/rectal frequency/urgency may be managed with diphenoxylate or loperamide. Bladder irritation may be mitigated with phenazopyridine. Urinary frequency/urgency can be managed with anticholinergic agents or alpha-blockers such as tamulosin. Erectile dysfunction can be managed with phosphodiesterase (PDE) inhibitors such as sildenafil.

4.4.1.9. Quality of Life Outcomes Related to Radiation Treatment
All patients will be given quality of life and symptom monitoring questionnaires including AUA symptom score sheet and the SHIM score sheet. The questionnaries will be given at the radiation consult visit and at follow up visits as part of the standard of care treatment.

4.4.2 SURGICAL PROCEDURES
4.4.2.1 Gold fiducial marker placement.
Gold seeds will be placed under ultrasound guidance in the prostate of patients as fiducial markers for radiation therapy prior to commencement of EBRT. Fiducial marker placement is standard of care for patients undergoing EBRT and will be performed following standard techniques.

4.4.2.2. Prostate biopsies.
Standard 12-core biopsies for diagnosis will be performed under transrectal ultrasound (TRUS) guidance at study entry on subjects who have not yet undergone diagnostic biopsy for prostate cancer. At the same time, up to 6 additional targeted core biopsy research samples will be obtained for purposes of the correlative studies described in Section 9.0. Subjects who have already undergone diagnostic prostate biopsies at the time of screening will undergo repeat

TRUS-guided biopsy to obtain up to 6 core biopsy samples for correlative studies at the time of study enrollment. TRUS-guided biopsy is performed as standard of care for the diagnosis and risk-stratification of patients with prostate cancer. A second set of up to -6 targeted research biopsy samples will be obtained under TRUS-guidance at the time of gold seed fiducial marker placement in all subjects enrolled in the study.

Biopsies will be formed using the Koelis Urostation with PROMAP-MR software. This approved, commercially available imaging and biopsy technology permits 3-dimensional transrectal ultrasound and MRI fusion to target tumor tissue within the prostate for biopsy, and will permit repeat biopsy of the same sites. The Urostation is a mobile software platform which can improve the prostate biopsy procedure with 3D imaging and image fusion capabilities. The Urostation connects to an ultrasound scanner equipped with a 3D transrectal probe. It receives stores and processes 3D Dicom images of the prostate as the physician performs the biopsy procedure. Organ-Based Tracking is an automatic 3DTRUS/3DTRUS registration based on the organ position within the image. This registration is robust to prostate and patient motion. The MRI/3DTRUS fusion is elastic since prostate deformation is corrected for optimal precision using a morphological contouring method. One significant advantage of this system is allowing mapping of biopsies as the Urostation guides and records each biopsy. It also allows real-time feedback on the targeted prostate region with an overlay of prior biopsy locations so a rebiopsy can be performed accurately of the region of interest.

4.5 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment may continue for up to 36 months or until:

- Disease progression
- Inter-current illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Subject decides to withdraw from the study, **OR**
- General or specific changes in the patient's condition render the subject unacceptable for further treatment in the judgment of the investigator".

4.6 Duration of Follow Up

Subjects will be followed for **12 months after initiation of treatment** with enzalutamide plus GnRH agonist or until death, whichever occurs first. Subjects removed from treatment for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. Subjects will be followed at monthly (every 28 +/- 3 days) visits for 12 months from the time of initiation of treatment with enzalutamide plus GnRH agonist. Total treatment will be for up to 36 months. The frequency of the follow up visits will be at the discretion of the investigator up to 36 months.The duration of treatment with the study agent enazalutamide will be for the initial 6 months, and follow up on study will continue for an additional 6 months following completion of enzalutamide.

4.7 Removal of Subjects from Protocol Therapy

Subjects will be removed from therapy when any of the criteria listed in Section 5.5 apply. Notify the Principal Investigator, and document the reason for study removal and the date the subject was removed in the Case Report Form. The subject should be followed-up per protocol.

4.8 Subject Replacement

If a subject is withdrawn from the study prior to the initiation of radiation therapy for reasons other than AEs as outlined in tables 4.2.1 and 4.2.2, an additional subject may be added to the study.

5.0 STUDY PROCEDURES

5.1 Screening/Baseline Procedures

Assessments performed exclusively to determine eligibility for this study will be done only after obtaining informed consent. Assessments performed for clinical indications (not exclusively to determine study eligibility) may be used for baseline values even if the studies were done before informed consent was obtained.

All screening procedures **with the exception of a bone scan, must be performed within - 56** days prior to registration unless otherwise stated. A bone scan must be performed within – 90 days prior to registration. The screening procedures include:

5.1.1 *Informed Consent*

5.1.2 *Medical history*

Complete medical and surgical history, history of infections

5.1.3 *Demographics*

Age, gender, race, ethnicity

5.1.4 *Review subject eligibility criteria*

5.1.5 *Review previous and concomitant medications*

5.1.6 *Physical exam including vital signs, height and weight*

Vital signs (temperature, pulse, respirations, blood pressure), height, weight

5.1.7 *Performance status*

Performance status evaluated prior to study entry .

5.1.8 *Adverse event assessment*

Baseline adverse events will be assessed. See section 6 for Adverse Event monitoring and reporting.

5.1.9 *Hematology*

5.1.10 *Blood draw for baseline blood counts, serum chemistries, PSA, and testosterone:*

Complete blood count with automated or manual differential. Comprehensive metabolic panel (CMP) to include: albumin, alkaline phosphatase, ALT/SGPT, AST/SGOT, BUN, creatinine, electrolytes (sodium, potassium, calcium, chloride, bicarbonate), glucose, and total bilirubin. PSA. Serum total testosterone.

5.1.11 *Tumor assessment and imaging*

5.1.12.1 For patients with prostatic adenocarcinoma histologically confirmed prostatic adenocarcinoma, imaging assessment will include CT of the abdomen and pelvis OR pelvic MRI; PA/lateral chest x-ray OR chest CT; and bone scan. CT, MRI, and CXR must be performed within 56 days of enrollment. Bone scan must be performed within 90 days of enrollment. At enrollment, these subjects will undergo a research MRI of the prostate with TRUS-guided targeted prostate tumor biopsies. For patients who have not yet undergone CT abdomen/pelvis or MRI of the pelvis but are otherwise eligible for the trial, the

mandated research sequences from the prostate MRI may be obtained concurrently with the required standard of care MRI.

5.1.12.2 For subjects who have not yet undergone diagnostic prostate biopsies to confirm the presence of prostatic adenocarcinoma at the time of study enrollment, but who otherwise meet eligibility criteria (as defined in section 3.1.1: serum PSA > 20 ng/dL and/or clinical T3 disease by digital rectal exam), standard of care diagnostic and research biopsies of the prostate will be performed simultaneously under ultrasound guidance. Subjects will undergo MRI of the pelvis, bone scan, and CXR (PA and lateral)or chest CT at enrollment. MRI of the pelvis must be performed prior to prostate biopsy.

5.2 Procedures During Treatment

Subjects will receive treatment in the following phases:

- Neo-adjuvant: Subjects will receive treatment with enzalutamide plus GnRH agonist for 8-10 weeks prior to the initiation of EBRT
- Concurrent: Subjects will continue to receive treatment with enzalutamide plus GnRH agonist for the duration of EBRT (8-10 weeks)
- Adjuvant: Subjects will receive treatment with enzalutamide plus GnRH agonist following completion of EBRT until a total duration of 24 weeks of enzalutamide plus GnRH agonist treatment has been completed
- Maintenance: Subjects will continue to receive GnRH agonist therapy following completion of treatment with enzalutamide to achieve a total of 24 - 36 months of GnRH agonist treatment.
- During the neo-adjuvant, concurrent, and adjuvant phases, subjects will receive treatment with enzalutamide plus GnRH agonist therapy for six 28 day cycles for a total duration of 24 weeks.

5.2.1 Prior to Each Treatment Cycle During Neo-Adjuvant, Concurrent, and Adjuvant phases (weeks 1-24):

- Physical exam, vital signs
- Hematology
- Serum chemistries
- Toxicity evaluation

5.2.2 Day – 56 to – 14:

- Procedure:
  - MRI pelvis
  - TRUS-guided prostate biopsies

5.2.3 Weeks 7-11

- MRI pelvis
- Fiducial marker placement and TRUS-guided targeted prostate biopsies

5.2.4 Every 4 weeks during weeks 28-48 of Maintenance Phase:

- Physical exam, vital signs
- Hematology
- Chemistries
- Toxicity evaluation

5.2.5 Every 8 weeks during weeks 1-48:

- PSA, testosterone

5.3 Follow-up Procedures

Subjects will be followed every 4 weeks(+/- 3 days) after completion of (or early withdrawal from) study treatment until 48 weeks (12 months) after initiation of enzalutamide plus GnRH agonist. Following completion of the study, subjects will continue with maintenance therapy with GnRH agonist for up to 36 months total treatment time as standard of care and will be monitored at the discretion of the treating provider according to standard of care.

5.4 Time and Events Table

	Pre-study Days -56 to - 14	Day 1	Every 4 weeks (weeks 4-48)	Every 8 Weeks (weeks 8-48)	Weeks 8-10	End of study visit
Assessment						
Informed Consent	X					
History and PE	X	X	X			X
Performance Status	X	X	X			X
Toxicity Evaluations		X	X			X
CBC	X	X*	X			
CMP	X	X*	X			
PSA, testosterone	X	X*		X		
CXR PA/lateral or CT chest	X					
Bone scan	X#					
CT abd and pelvis	x					
MRI pelvis	X				X	
TRUS-guided prostate biopsy	X				X	

*These tests will be repeated at day 1 if the pre-screening tests were done greater than 28 days before Day 1.

Bone scan will be performed during pre-study days – 90 to – 14.

5.5 Removal of Subjects from Study

Subjects can be taken off the study treatment and/or study at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuation will be documented and may include:

5.5.1 Subject voluntarily withdraws from treatment (follow-up permitted);

5.5.2 Subject withdraws consent (termination of treatment and follow-up);

5.5.3 Subject is unable to comply with protocol requirements;

5.5.4 Subject demonstrates disease progression (unless continued treatment with study drug is deemed appropriate at the discretion of the investigator);

5.5.5 Subject experiences toxicity that makes continuation in the protocol unsafe;

5.5.6 Treating physician judges continuation on the study would not be in the subject's best interest;

5.5.8 Development of second malignancy (except for basal cell carcinoma or squamous cell carcinoma of the skin) that requires treatment, which would interfere with this study;

5.5.9 Lost to follow-up. If a research subject cannot be located to document survival after a period of 12 months, the subject may be considered "lost to follow-up." All attempts to contact the subject during the 12 months must be documented and approved by the Data Monitoring Committee.

6.0 Measurement of Effect

6.1 Antitumor Effect- Solid Tumors

Patients with high-risk localized or locally-advanced prostate cancer are eligible for enrollment in this study. Patients with high-risk localized prostate cancer by definition will not have measurable disease by RECIST. Patients also may have radiographic evidence of metastasis in regional lymph nodes (N1 disease as defined by the National Comprehensive Cancer Network Prostate Cancer Guideline Verson 3.2012) at the discretion of the treating physicians, if regional lymph nodes can be included in the planned radiation field. RECIST will not be used to monitor these regional lymph nodes for treatment response. Rather, in all patients, treatment response will be monitored by serum prostate specific antigen (PSA). The PSA nadir with EBRT combined with androgen deprivation therapy is known to vary. Following RTOG-ASTRO Phoenix Consensus, a PSA rise by 2 ng/mL or more above the nadir PSA is the standard definition for biochemical failure after EBRT with or without ADT. As the primary endpoint of this study is safety and tolerability as measured up to 12 months following initiation of combined enzalutamide and GnRH agonist therapy, it is expected that subjects will remain under treatment with maintenance GnRH agonist therapy at the completion of their time on study. Consequently, it is anticipated that subjects will not experience a PSA rise suggestive of disease progression as defined by RTOG-ASTRO Phoenix Consensus during the study enrollment period. Should subjects experience any PSA rise on 2 or more consecutive measurements while enrolled in the study, this will be considered evidence of disease progression. Subjects will undergo research MRI of the pelvis to quantify local response following 8 weeks of combined enzalutamide plus GnRH agonist treatment for research purposes.

6.1.1 Definitions

Evaluable for toxicity. All subjects will be evaluable for toxicity from the time of their first treatment with study drug.

6.1.2 Methods for Evaluation of Measurable Disease: Magnetic Resonance Imaging (MRI) of the prostate and pelvis

6.1.2.1 Patient preparation

The participants will undergo prostate MRI following the standard institutional procedure. If patients have had prostate biopsies, a minimum of 3 weeks is recommended between the date of the MRI exam and the previous biopsy to reduce the influence of post-biopsy changes in diagnostic accuracy.[15] Patients will be asked to refrain from ejaculation for 3 days preceding the examination to maximize distention of the seminal vesicle.[16]

6.1.2.2 Image acquisition

Patients will undergo an MR examination with our routine clinical protocol using a 3T MRI system (Philips Medical Systems, Cleveland, OH) and a pelvic phased-array coil. Endorectal coil will not be used. The patient is imaged in the supine position and artifacts due to bowel peristalsis are minimized with sublingual administration of 0.250 mg of hyoscyamine sulfate (Levsin). Our protocol includes T2-weighted fast spin-echo images in the transverse, sagittal, and coronal planes; axial diffusion-weighted transverse single shot echo planar image (with ADC map); T1-weighted gradient-echo images acquired before, during, and following the intravenous injection of a single dose of a gadolinium-based contrast as it is done for our clinical prostate MRI protocol. Additional pulse sequences such as arterial spin labeling (ASL), blood oxygen level dependent (BOLD), and others might be incorporated into the MRI protocol for additional research purposes; the total examination time will not exceed 1.5 hours and the amount of intravenous contrast administered will be similar to the dose given for clinical prostate MRI examinations. If at any point during the study the participant indicates a desire to withdraw, the exam will be interrupted.

6.1.2.3 Potential risks associated with MRI

Patients will be screened for MRI contraindications and these will be exclusion criteria for this study. Safety rules will be strictly enforced and followed by the research study team, MRI technologists, and the study subjects themselves. During the MRI scanning process, the study team is in constant two-way communication with the participant and can remove him from the magnet immediately as needed. The MRI personnel are trained to respond to emergency situations, further supported by an institutional Rapid Response Team should an adverse event arise during the study visit. Standard radiofrequency coils provided by manufacturers of MRI machines will be used in this study. The risks associated with the use of these coils are minimal. However, with any RF coil, there is a small risk of skin burns if the coil is used improperly or is malfunctioning. The MRI staff at UT Southwestern Medical Center is fully trained in the proper use of these devices.

Subjects will be screened for renal impairment prior to administration of gadolinium-based contrast agents as it is routinely done in the Radiology Department at UT Southwestern. A serum creatinine determination within 30 days prior to the research MRI examination must be available to calculate an estimated glomerular filtration rate (eGFR). An eGFR calculation may be repeated closer to the MRI appointment if the patient's medical condition has changed. An eGFR ≥45 mL/min/1.73m2 is considered acceptable for administration of a standard weight-based dose of intravenous contrast for research purposes.[17]

We do not anticipate significant psychological stress related to this study procedure. Patients will be able to refuse to answer any of the questions, take a break or stop their participation in this study at any time.

6.1.2.4 Image interpretation

MR images will be interpreted by a single radiologist. The following criteria for tumor identification are widely accepted in the literature and are used in routine clinical practice: round or ill-defined, low-signal-intensity focus in the peripheral zone; or homogeneous signal mass with indistinct margins in the transition zone on T2-weighted imaging; high signal intensity on DWI and low ADC values when compared to the remainder of the prostate tissue.[18] In addition, dynamic contrast-enhanced images will be analyzed using a FDA-approved software for

pharmacokinetic evaluation (VividLook with Versavue Enterprise, iCAD, Nashua, NH). Suspicious areas for tumor are defined as those with increased relative peak enhancement and subsequent decrease in signal intensity—"rapid wash-in and wash-out pattern"). Color-coded map will be generated to highlight areas with high microvascular permeability and low to medium extracellular volume fraction.[19]

6.1.2.5 MRI-US image fusion

Anatomic co-registration of biopsy samples and MRI findings are essential in this proposal. Transrectal ultrasound images will be fused with initial MRI performed prior to therapy using a specialized device (UroStation; Koelis, Grenoble, France). This technology allows 3-dimensional anatomic co-registration of volumetric transrectal ultrasound (TRUS) and MRI acquisitions. Once the TRUS and MRI volumes are co-registered, the urologist can use the real-time fusion dataset to perform routine sextant biopsies and also target biopsies directly to the suspicious areas on MRI. Furthermore, this system allows for saving a 3D map of the prostate with the biopsy tracts of the needle biopsies and data from pathologic analysis, once it becomes available, can be loaded into the system for further analysis. Because these 3D maps can be loaded into the biopsy US unit prior to performing the follow-up biopsy after treatment, we will use this capability to facilitate the anatomic coregistration of the initial pre-treatment biopsies and the repeat biopsy performed after treatment.[20]

6.1.2.6 Evaluation of changes in tumor volume and tumor activity

Tumor volume will be assessed using a planimetric approach.[21] On each MRI prostate section the outline of suspicious areas will be traced on the computer screen and the surface area will be calculated. After counting the number of separate foci and defining the most representative site of origin, tumor foci volume will be calculated by multiplying the sum of the suspicious surface areas by the slice thickness (usually 2-3 mm).

Tumor activity will be assessed using apparent diffusion coefficient (ADC) derived from diffusion-weighted imaging, which has been shown to correlate with Gleason score and percentage of tumor on core biopsy (as surrogates for tumor aggressiveness).[22,23] ADC values will be derived by drawing a region of interest on the confirmed tumors, and expressed as mean values and histograms.

6.2 Safety/tolerability

Analyses will be performed for all subjects having received at least one dose of study drug. The study will use the CTCAE version 4.0 for reporting of non-hematologic adverse events (http://ctep.cancer.gov/reporting/ctc.html) and modified criteria for hematologic adverse events .

7.0 ADVERSE EVENTS

7.1 Experimental Therapy

For the most recent safety update, please refer to the current Investigator's Brochure or Study Agent Prescribing Information.

7.1.1 Contraindications: Enzalutamide is contraindicated in pregnancy.

7.1.2 Special Warnings and Precautions for Use: Seizure occurred in 0.9% of patients who received enzalutamide in a completed phase III trial. There is no clinical trial experience with enzalutamide in patients who have had a seizure, in patients with predisposing risk factors for

seizure, or in patients using concomitant medications that may lower the seizure threshold. Patients with a history of seizure or with predisposing risk factors for seizure are ineligible to participate in this study.

7.1.3 Interaction with other medications:

In vitro studies show that enzalutamide and/or metabolite M2 are potential inhibitors of CYP2C8 and CYP2C19 with lesser potential for inhibitory effects on CYP2B6 and CYP2C9. Enzalutamide is a moderate CYP2C9 and CYP2C19 inducer in humans. For this study, substrates of CYP2B6, CYP2C8, CYP2C9, and CYP2C19 that have a narrow therapeutic index (e.g., paclitaxel, phenytoin, warfarin, omeprazole) are prohibited during the period of enzalutamide administration.

In vitro studies show that enzalutamide is an inducer of CYP3A4. Induction of CYP3A occurs via activation of the nuclear pregnane X receptor (PXR), which is expected to result in co-induction of CYP2C. Co-administration of enzalutamide with CYP3A or CYP2C substrates may reduce oral bioavailability and/or accelerate elimination of these substrates. Enzalutamide is a strong CYP3A4 inducer in humans. For this study, narrow therapeutic index drugs that are metabolized by CYP3A4 (e.g., alfentanil, cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus and tacrolimus) are prohibited.

In vitro studies show that enzalutamide is metabolized by CYP2C8 and CYP3A4/5. Strong inhibitors or inducers of these enzymes may affect enzalutamide exposures. Co-administration of a strong CYP2C8 inhibitor (gemfibrozil) increased the composite area under the plasma concentration-time curve (AUC) of enzalutamide plus N-desmethyl enzalutamide in healthy volunteers. For this study, co-administration of enzalutamide and strong CYP2C8 inhibitors (e.g., gemfibrozil) is prohibited. Co-administration of a strong CYP3A4 inhibitor (itraconazole) increased the composite AUC of enzalutamide plus N-desmethyl enzalutamide by 1.3 fold in healthy volunteers. For this study, co-administration of enzalutamide and strong inhibitors of CYP3A4/5 (e.g., clarithromycin, itraconazole, ketoconazole) are prohibited.

Use caution when co-administering strong inducers of CYP2C8 (e.g., rifampin) or CYP3A4/5 (e.g., carbamazepine, phenytoin, rifampin, St. John's wort) during enzalutamide treatment, as enzalutamide concentrations may decrease.

In vitro studies show that enzalutamide and metabolite M2 are potential inhibitors of the efflux transporter P-gp. Co-administration of enzalutamide with P-gp substrates may increase the plasma concentrations of the P-gp substrate. Use caution when co-administering sensitive P-gp substrates (e.g., colchicine, dabigatran etexilate, digoxin) during enzalutamide treatment.

7.1.4 Adverse Reactions

The most common adverse reactions (≥5%) reported in patients receiving enzalutamide in the completed randomized clinical trial, which included patients with metastatic castrate-resistant prostate cancer who previously received docetaxel chemotherapy, included asthenia/fatigue, back pain, diarrhea, arthralgia, hot flush, peripheral edema, musculoskeletal pain, headache, upper respiratory infection, muscular weakness, dizziness, insomnia, lower respiratory infection, spinal cord compression and cauda equine syndrome, hematuria, paresthesia, anxiety, and hypertension. Grade 3 and higher adverse reactions were reported among 47% of enzalutamide-treated patient and 53% of placebo-treated patients.

PRES is a rare, reversible, neurological disorder which can present with rapidly evolving symptoms including seizure, headache, confusion, blindness, and other visual and neurological

disturbances, with or without associated hypertension. A diagnosis of PRES requires confirmation by brain imaging, preferably magnetic resonance imaging (MRI). Discontinuation of enzalutamide in patients who develop PRES is recommended. Patients should be informed to contact the study physician as soon as possible if they experience rapidly worsening symptoms possibly indicative of PRES such as seizure, headache, confusion, reduced eye sight, or blurred vision.
Three reported PRES cases include one clinical trial case assessed as unrelated to enzalutamide treatment and two post-marketing cases assessed as possibly associated with enzalutamide.
One confirmed case of PRES, assessed as unrelated to enzalutamide, was reported in Study S-3100-1-01 (first in man dose escalation study in castration-resistant prostate cancer patients). The onset of PRES symptoms occurred approximately 27 days after discontinuation of enzalutamide, and approximately 19 days after initiating treatment with an investigational insulin-like growth factor (IGF) antibody (onset - 3 days after the second dose). PRES was likely associated with the investigational IGF antibody based on temporal relationship and positive dechallenge.
Two post marketing cases of confirmed PRES were identified from the global safety database (estimated cumulative post marketing exposure of 17,704 patient treatment years as of 30August2014). In the first case, reported symptoms (confusional syndrome and aphasia; symptom onset on treatment day 27) and MRI findings were consistent with a diagnosis of PRES. PRES was assessed as possibly associated with enzalutamide treatment in this case based on a plausible temporal relationship and the lack of an alternative etiology. The patient received acute treatment with intravenous corticosteroids. After discontinuing enzalutamide, reported symptoms resolved and a follow-up cerebral MRI revealed regression of abnormalities, indicative of positive dechallenge. In the second case, reported symptoms (altered mental status, confusion, and word-finding difficulties; with a blood pressure of 188/97 mm Hg; symptom onset on treatment day 98) and MRI findings were consistent with a diagnosis of PRES. PRES was assessed as possibly associated with enzalutamide treatment in this case based on a plausible temporal relationship and the lack of an alternative etiology. The patient was treated with dexamethasone and an antihypertensive regimen of hydralazine and carvedilol. After discontinuing enzalutamide, reported symptoms resolved and follow-up cerebral MRI finding showed significant improvement, indicative of positive dechallenge.

7.2 Adverse Event Monitoring

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial. Additionally, certain adverse events must be reported in an expedited manner to allow for optimal monitoring of subject safety and care.

All subjects experiencing an adverse event, regardless of its relationship to study drug, will be monitored until:

- the adverse event resolves or the symptoms or signs that constitute the adverse event return to baseline;
- any abnormal laboratory values have returned to baseline;
- there is a satisfactory explanation other than the study drug for the changes observed; or
- death.

7.3 Definitions

7.3.1 Definition of Adverse Event

An adverse event (AE) is any untoward medical occurrence in a subject receiving study treatment and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign

(including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an experimental intervention, whether or not related to the intervention.

7.3.2 Severity of Adverse Events

All non-hematologic adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The CTCAE v4 is available at http://ctep.cancer.gov/reporting/ctc.html

If no CTCAE grading is available, the severity of an AE is graded as follows:

Mild (grade 1): the event causes discomfort without disruption of normal daily activities.
Moderate (grade 2): the event causes discomfort that affects normal daily activities.
Severe (grade 3): the event makes the patient unable to perform normal daily activities or significantly affects his/her clinical status.
Life-threatening (grade 4): the subject was at risk of death at the time of the event.
Fatal (grade 5): the event caused death.

7.3.3 Serious Adverse Events

A “serious” adverse event is defined in regulatory terminology as any untoward medical occurrence that:

Results in death.
If death results from (progression of) the disease, the disease should be reported as event (SAE) itself.

Is life-threatening.
(the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe).

Requires in-patient hospitalization or prolongation of existing hospitalization for ≥ 24 hours.

Results in persistent or significant disability or incapacity.

Is a congenital anomaly/birth defect

Is an important medical event
Any event that does not meet the above criteria, but that in the judgment of the investigator jeopardizes the subject, may be considered for reporting as a serious adverse event. The event may require medical or surgical intervention to prevent one of the outcomes listed in the definition of “Serious Adverse Event“.
For example: allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that may not result in hospitalization; development of drug abuse or drug dependency.

7.3.4 Unanticipated Problems:

The term “unanticipated problem” is found, but not defined in the regulations for the Protection of Human Subjects at 45 CFR 46, and the FDA regulations at 21 CFR 56. Guidance from the regulatory agencies considers unanticipated problems to include any incident, experience, or outcome that meets ***each*** of the following criteria:

- unexpected; ***and***
- related *or possibly related* to participation in the research; ***and***
- suggests that the research *places subjects or others at a greater risk of harm* (including physical, psychological, economic, or social harm) *than was previously known or recognized.*

Follow-up

All adverse events will be followed up according to good medical practices.

7.3.5 Reporting

Local events requiring expedited reporting, are submitted to the UTSW IRB through the UTSW eIRB and to the SCC DSMC Coordinator. Hardcopies or electronic versions of the eIRB report; the NCI ADEERS, FDA Form #3500A forms, or other sponsor forms, if applicable; and/or any other supporting documentation available should be forwarded to the DSMC Coordinator. The DSMC Coordinator forwards the information onto the DSMC Chairman who determines if immediate action is required. Follow-up eIRB reports, and all subsequent SAE documentation that is available are also submitted to the DSMC Chair who determines if further action is required.

The DSMC Chairperson reviews all serious adverse events within upon receipt from the DSMC Coordinator. The DSMC Chairperson determines whether action is required and either takes action immediately, convenes a special DSMC session (physical or electronic), or defers the action until a regularly scheduled DSMC meeting.

Telephone reports to: Kevin Courtney, MD, PhD at 214-648-4180 or Allison Beaver, RN at 214-645-8787 UTSW SCC Data Safety Monitoring Committee Coordinator (if fax report is not available) within 1 working day to 214-648-7097.
Written reports to: Kevin Courtney, MD, PhD, or Allison Beaver, RN at 214-645-8766 UTSW SCC Data Safety Monitoring Committee Coordinator Email: SCCDSMC@utsouthwestern.edu Fax: 214-648-7018 or deliver to NB 2.418 UTSW Institutional Review Board (IRB) Submit via eIRB with a copy of the final sponsor report as attached supporting documentation

1. Unexpected Adverse Events

Non-serious adverse events which are classified as both unexpected (in terms of nature, severity and frequency) and possibly related require reporting to the UTSW IRB within 10 working days of PI awareness.

2. SAEs

i. Local unanticipated problems require expedited reporting, and are submitted to the UTSW IRB through the UTSW eIRB and to the SCC DSMC Coordinator. Hardcopies or electronic versions of the eIRB report; FDA Form #3500A forms, or other sponsor forms, if applicable; and/or any other supporting documentation available should be forwarded to the DSMC Coordinator. The DSMC Coordinator forwards the information onto the DSMC Chairman who determines if immediate action is required. Follow-up eIRB reports, and all subsequent SAE documentation that is available are also submitted to the DSMC Chair who determines if further action is required.

ii. All local serious adverse events which occur on research subjects on protocols for which the SCC is the DSMC of record require reporting to the DSMC regardless of whether IRB reporting is required. Hardcopies or electronic versions of the FDA Form #3500A forms, or other sponsor forms, if applicable; and/or any other supporting documentation available should be forwarded to the DSMC Coordinator.

iii. If the event occurs on a multi-institutional clinical trial coordinated by the Cancer Center, the DOT Manager or lead coordinator ensures that all participating sites are notified of the event and resulting action, according to FDA guidance for expedited reporting. DSMC Chairperson reviews all serious adverse events within upon receipt from the DSMC Coordinator. The DSMC Chairperson determines whether action is required and either takes action immediately, convenes a special DSMC session (physical or electronic), or defers the action until a regularly scheduled DSMC meeting.

	Telephone reports to: Kevin Courtney, MD, PhD at 214-648-4180 or Allison Beaver at 214-645-8787 UTSW SCC Data Safety Monitoring Committee Coordinator (if fax report is not available) within 1 working day to 214-648-7097.
	Written reports to: Fax – Kevin Courtney, MD, PhD or Allison Beaver, RN at 214-645-8766. UTSW SCC Data Safety Monitoring Committee Coordinator Email: SCCDSMC@utsouthwestern.edu Fax: 214-648-7018 or deliver to NB 2.418 UTSW Institutional Review Board (IRB) Submit via eIRB with a copy of the final sponsor report as attached supporting documentation

3. Unanticipated Problems

Unanticipated problems, including those that occur as non-local events, require reporting to the UTSW IRB within 10 working days of PI awareness of the event.

For further guidance for Investigators regarding safety reporting requirements for INDs and BA/BE studies, refer to FDA Draft Guidance document:
http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM227351.pdf

7.4 Steps to Determine If an Adverse Event Requires Expedited Reporting

Step 1: Identify the type of adverse event using the NCI Common Terminology Criteria for Adverse Events (CTCAE v4).

Step 2: Grade the adverse event using the NCI CTCAE v4.

Step 3:Determine whether the adverse event is related to the protocol therapy
Attribution categories are 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.
- Unrelated – The AE *is clearly NOT related* to the study treatment.

Note:This includes all events that occur within 30 days of the last dose of protocol treatment. Any event that occurs more than 30 days after the last dose of treatment and is attributed (possibly, probably, or definitely) to the agent(s) must also be reported accordingly.

Step 4: Determine the prior experience of the adverse event.
Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is not listed in:

- the current known adverse events listed in the Agent Information Section of this protocol;
- the drug package insert;
- the current Investigator's Brochure

7.5 Reporting Requirements for Adverse Events

- The FDA should be notified within 7 business days of any unexpected fatal or life-threatening adverse event with possible relationship to study drug, and 15 business days of any event that is considered: 1) serious, 2) unexpected, and 3) at least possibly related to study participation.

INSERT PROPER REPORTING PROCEDURES REQUIRED BY SPONSOR

- Please email or fax the SAE Worksheet to:
- Astellas Pharma Global Development – United States
- Email: Safety-us@us.astellas.com
- Fax number: (847) 317-1241
- Follow-up information for the event should be sent promptly (within 7 days) as necessary.
- Full details of the SAE should also be recorded on the medical records and on the CRF.
- The following minimum information is required:
- ISN/Study number
- Subject number, sex and age
- The date of report

- A description of the SAE (event, seriousness of the event)
- Causal relationship to the study drug
- The period for SAE reporting after the last intake of the study drug should be specified.
- Expedited Safety Reports: An unexpected adverse event is one for which the nature or severity is not consistent with the current Investigator's Brochure. The Sponsor will make this assessment for all reported serious adverse events. An adverse event which is serious, related, and unexpected may be termed a Suspected Unexpected Serious Adverse Reaction (SUSAR). The Investigator will ensure that all relevant information is provided as soon as possible to Astellas in order that the Sponsor may meet their obligations to report any SUSAR.

7.5.1 Routine Reporting

- All other adverse events- such as those that are expected, or are unlikely or definitely not related to the study participation- are to be reported annually as part of regular data submission.

7.6 Unblinding Procedures

This is an open-label study. Unblinding procedures will not be applicable.

7.7 Stopping Rules

Based on experience from a completed phase III study, enzatlutamide is generally well tolerated when given in combination with a GnRH agonist. The most prominent safety concerns are primarily related to the risk of seizures. No completed study has examined the combination of enzalutamide with a GnRH agonist and concurrent radiation. The general tolerability of this combination is anticipated to be similar to that of radiation with concurrent GnRH agonist therapy. However, the absence of unexpected adverse events must be verified and is the primary endpoint of this study. Adverse events will be measured by NCI CTCAE criteria and will be monitored and recorded for a period of 12 months from initiation of the combination of enzalutamide plus GnRH agonist. Enrollment in the trial will be halted if unacceptable levels of toxicity are found as defined by 3 or more patients withdrawing from the study due to AEs.

8.0 DRUG INFORMATION

8.1 Enzalutamide

- Other names for the drug: Xtandi, MDV3100
- Classification - type of agent: oral androgen receptor inhibitor
- Mode of action: inhibits androgen-induced receptor activation (binding of androgens to ARs in the cytosol), inhibits nuclear translocation of activated ARs, and inhibits the association of the activated AR with chromatin
- Storage and stability: Enzalutamide capsules should be stored at 20 C to 25 C (68 F to 77 F) in a dry place. Excursions are permitted from 15 C to 30 C (59 F to 86 F).
- Protocol dose: Subjects will take four 40 mg capsules (160 mg) daily. Patients will be given one bottle of 120 capsules at every 4 week follow up visit.
- Route of administration for this study: oral

- Availability: provided by sponsor free of charge

- Side effects: Adverse events associated with enzalutamide that are most likely to occur in this study include asthenia/fatigue, back pain, diarrhea, arthralgia, hot flush, peripheral edema, musculoskeletal pain, headache, upper respiratory infection, muscular weakness, dizziness, insomnia, lower respiratory infection, hematuria, paresthesia, anxiety, and hypertension. Please refer to the Xtandi (enzalutamide) package insert for a comprehensive list of adverse events.

- Nursing implications:

8.2 GnRH agonist – For the purposes of this study we are only using Lupron. Additonal GnRH agonists are listed below for informational purposes only.

- Description: GnRH agonists are long-acting analogs of the native GnRH peptide and are effective at reducing serum testosterone. Analogs approved by the FDA.
- Supply: Commercially available.
- Storage: GnRH analogs should be stored as directed by the commercial supplier.
- Administration: GnRH analogs are administered with a variety of techniques, including subcutaneous insertion of a solid plug in the abdominal wall (Zoladex), intramuscular injection (Lupron), subcutaneous injection (Eligard), or insertion of a long-acting cylinder that slowly Releases the agent (Viadur). The manufacturer's instructions should be followed.
- Toxicity: Consult the package insert for comprehensive toxicity information. Class-related toxicity is generally a manifestation of the mechanism of action and due to low testosterone levels. In the majority of patients testosterone levels increased above baseline during the first week, declining thereafter to baseline levels or below by the end of the second week of treatment. The most common side effect of GnRH analogs is vasomotor hot flashes; edema, gynecomastia, bone pain, thrombosis, and gastrointestinal disturbances have occurred. Potential exacerbations of signs and symptoms during the first few weeks of treatment is a concern in patients with vertebral metastases and/or urinary obstruction or hematuria which, if aggravated, may lead to neurological problems such as temporary weakness and/or paresthesia of the lower limbs or worsening of urinary symptoms. Other side effects include impotence and loss of libido, weight gain, depression, dizziness, loss of bone density, anemia,increased thirst and urination, unusual taste in the mouth, skin redness or hives, pain at injection site, and muscle mass and strength loss, hair changes, penile length and testicular volume loss, increased cholesterol, hypertension, diabetes exacerbation, emotional lability,nausea, vomiting, and rarely allergic generalized rash and difficulty breathing.

8.2.1 Return and Retention of Study Drug

Unused study drug (enzalutamide) will be documented and returned to the Simmons Comprehensive Cancer Center Pharmacy for destruction per Standard Operating Procedures

8.2.2 Subject compliance with the study agent, enzalutamide, will be monitored through use of a pill diary provided to each subject enrolled in the study. A new pill diary will be provided at every 4 week follow up visit for the duration of the administration of enzalutamide on the study.

9.0 CORRELATIVES/SPECIAL STUDIES

The goal of the planned laboratory correlative studies is to evaluate the proposed mechanism of action of enzalutamide plus GnRH agonist on patient tumor samples. The proposed mechanism of action of enzalutamide has been established in pre-clinical studies. However, to date the molecular mechanism of action has not been reported in primary prostate cancer tumors from

patients. The purpose of the proposed laboratory correlative studies is to examine androgen signaling inhibition by enzalutamide in primary tumor samples from patients. Submission of samples for correlative studies is mandatory for participation in this trial.

9.1 Sample Collection Guidelines

Up to 6 needle core biopsies will be collected using 18 gauge needles under TRUS-guidance at each of two occasions as described in Section 4.4.2.2. Biopsies will be performed at study entry and following 8 weeks of treatment with combined enzalutamide plus GnRH agonist. Samples will be submitted to pathology laboratory for diagnosis and to UTSW tissue bank. Samples will be labeled with the subject's de-identified study number and collection date and delivered for analysis to:

Dr. Nima Sharifi, MD
Cleveland Clinic and also to Dr. Payal Kapur and Dr. Yi Yin at UT Southwestern Medical Center.

9.2 Assay Methodology

9.2.1 Immunohistochemistry for Androgen receptor (AR) and Ki-67 will be done using routine immunohistochemical assay in a CLIA certified clinical laboratory at UTSW. The slides will be analysed under a light microscope to access cellular localization of the antibody. Tissue procured for diagnosis will be processed using routine tissue processing protocols followed for pathologic diagnosis.

9.2.2 Gene expression studies

Ligand-bound androgen receptor (AR) activity may be defined by the expression of androgen-responsive genes. *PSA*, *TMPRSS2* and *FKBP5* are among the most widely studied and validated among these genes.[24] RNA will be extracted from the collected prostate cancer tissue using the RNeasy system (Qiagen, Germantown, MD), and 1 µg RNA will be used in a reverse transcription reaction using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). Quantitative-PCR (qPCR) analysis will be performed in triplicate using previously published primers for *PSA, TMPRSS2, FKBP5* and for the housekeeping gene *large ribosomal protein P0* (*RPLP0*).[25] Absolute qPCR SYBR Low Rox Mix (Fisher, Waltham, MA) will be used in 25 µl final reaction volume, in 96-well plates. The thermocycling reaction will carried out in an ABI 7500 Real Time PCR machine (Applied Biosystems, Foster City, CA). Accurate quantitation of each mRNA will be achieved by normalizing the sample values to *RPLP0* and to vehicle treated cells. Samples will be labeled with the subject's de-identified study number and collection date and delivered for gene expression analysis to

Nima Sharifi, M.D.
Kendrick Family Endowed Chair for Prostate Cancer Research
Cleveland Clinic
Lerner Research Institute
Cancer Biology, NB40
9500 Euclid Avenue
Cleveland, OH 44195

9.2.3 Genomic sequence analysis of selected steroidogenic enzymes

The first and rate-limiting step for metabolic flux from adrenal dehydroepiandrosterone (DHEA) to dihydrotestosterone (DHT) is catalyzed by 3β-hydroxysteroid dehydrogenase/isomerase, which is encoded by two isoenzymes, *HSD3B1* and *HSD3B2*.[26] Our unpublished observations suggest that genomic alterations of one or both of these genes may contribute to the development of resistance to various hormonal therapies in prostate cancer. Genomic DNA will be prepared from collected samples (tumor and matched peripheral blood) using DNeasy Blood and Tissue Kit (QIAGEN,

Germantown, MD). PCR products of the promoter region, all exons, exon-intron junctions and the 3'-UTR will be sequenced to identify mutations in *HSD3B1* and *HSD3B2*. The primers and annealing temperature were described previously.[27] To sequence the 3' flanking region of *HSD3B1*, primer set (Forward: 5'-ATGTGGAGGGAGGTGTGAGT-3' and Reverse: 5'-ACGGAGATGGGTCTCTTCCA-3') will be used with an annealing temperature of 62°C. Genotyping PCR reaction (50 μl) consisted of 30-100 ng genomic DNA, 1 x PCR buffer with 0.2 mM dNTP, 0.2 μM of each primer, and 0.5 μl Phusion High-Fidelity DNA Polymerase (New England BioLabs Inc, Ipswich, MA). Tumor and germline sequences will be compared in each individual patient to assess for somatically acquired mutations. Samples will be labeled with the subject's de-identified study number and collection date and delivered for genomic sequence analysis to

Nima Sharifi, M.D.
Kendrick Family Endowed Chair for Prostate Cancer Research
Cleveland Clinic
Lerner Research Institute
Cancer Biology, NB40
9500 Euclid Avenue
Cleveland, OH 44195

9.3 Specimen Banking

The tissue used for diagnosis will be stored for 10 years in the CLIA certified clinical Pathology laboratory at UTSW. The tissue procured for research will be stored snap frozen in the UTSW tissue bank indefinitely or until used up. Serum and tissue may be used for future additional studies with specific consent from study subjects. If future use is denied or withdrawn by the subject, best efforts will be made to stop any additional studies and to destroy the specimens.

The specimens, DNA, and their derivatives may have significant therapeutic or commercial value. The Informed Consent form contains this information and informs the subject that there is the potential for financial gain by UT Southwestern, the investigator, and the study sponsors Medivation, Inc. and Astellas Pharma US, Inc.

The following information obtained from the subject's medical record may be provided to research collaborators when specimens are made available:

- Diagnosis
- Collection time in relation to study treatment
- Clinical outcome – if available
- Demographic data

10.0 STATISTICAL CONSIDERATIONS

10.1 Study Design/Study Endpoints

This is an open-label, single-arm, single-center prospective pilot study of enzalutamide plus GnRH agonist treatment combined with EBRT in the treatment of men with high-risk localized or locally advanced prostate cancer. Select patients with regional lymph node involvement (N1 disease) are also eligible to participate in this study.

Primary endpoint: to determine the safety and tolerability of combining enzlutamide plus a GnRH agonist with EBRT up to 12 months following initiation of treatment with enzalutamide plus GnRH agonist.

Secondary endpoints:
1. Molecular markers of androgen signaling and cell death in prostate biopsy samples

a. Inhibition of androgen-regulated gene expression (potential targets to include NDRG1, FKBP5, TMPRSS2, and PSA) by qRT-PCR
b. Inhibition of androgen receptor (AR) nuclear localization and enhancement of apoptotic cell death measured by immunohistochemistry (IHC)
c. Androgen metabolism in prostate biopsy samples

2. Serum PSA, testosterone, dehydroepiandrosterone (DHEA), and DHEA-sulfate (DHEA-S) following combination therapy with enzalutamide plus GnRH agonist therapy prior to initiation of radiation therapy
3. Changes in prostate tumor volume and activity following 2 months of combined enzalutamide plus GnRH therapy compared to baseline as measured by multi-parametric magnetic resonance imaging (MRI)

The trial will be halted if unacceptable levels of toxicity are found as defined by 3 or more patients withdrawing from the study due to AEs or if 3 or more patients experience SAEs.

10.2 Sample Size and Accrual

We propose to enroll ten subjects in this pilot study. The expected accrual rate is 7 subjects over approximately 4 months. The primary endpoint is safety and tolerability of enzalutamide when combined with GnRH agonist and radiation therapy. Descriptive statistics will be computed to assess safety and tolerability. The highest AE score for each category over the 12 month period following initiation of treatment with enzalutamide plus GnRH agonist will be reported for each study subject in the summary statistics. Secondary endpoints assess mechanisms of enzalutamide plus GnRH action on prostate cancer tissue and quantitative imaging of localized prostate cancer response to enzalutamide plus GnRH therapy. As this is a pilot study, statistical power has not been calculated to determine the sample size, and descriptive statistics will be used to report outcomes.

10.3 Data Analyses Plans

Primary endpoint: to determine the safety and tolerability of combining enzlutamide plus a GnRH agonist with EBRT up to 12 months following initiation of treatment with enzalutamide plus GnRH agonist. Data analysis plan: descriptive statistics to report the number and nature of AEs in all patients over 12 months.

Secondary endpoints:

1. Molecular markers of androgen signaling and cell death in prostate biopsy samples will be analyzed using descriptive statistics
    a. Inhibition of androgen-regulated gene expression (potential targets to include NDRG1, FKBP5, TMPRSS2, and PSA) by qRT-PCR
    b. Inhibition of androgen receptor (AR) nuclear localization and enhancement of apoptotic cell death measured by immunohistochemistry (IHC)
    c. Androgen metabolism in prostate biopsy samples
2. Serum PSA, testosterone, dehydroepiandrosterone (DHEA), and DHEA-sulfate (DHEA-S) following combination therapy with enzalutamide plus GnRH agonist therapy prior to initiation of radiation therapy will be reported using descriptive statistics for each patient.
3. Changes in prostate tumor volume and activity following 2 months of combined enzalutamide plus GnRH therapy compared to baseline as measured by multi-parametric magnetic resonance imaging (MRI) will be reported using descriptive statistics for each patient and summarized with mean change and standard deviation.

11.0 STUDY MANAGEMENT

11.1 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by the Conflict of Interest office. All investigators will follow the University conflict of interest policy.

11.2 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB must approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the subject will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the subject and the investigator is assured that the subject understands the implications of participating in the study, the subject will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the subject and by the person who conducted the informed consent discussion.

11.2 Registration Procedures

All subjects must be registered with the Urology Research Office before enrollment to study. Prior to registration, eligibility criteria must be confirmed with the (Research Office) Study Coordinator. To register a subject, call 214-645-8787 Monday through Friday, 9:00AM-5:00PM.

11.3 Data Management and Monitoring

The UTSW Simmons Cancer Center (SCC) Data Safety Monitoring Committee (DSMC) is responsible for monitoring data quality and patient safety for all UTSW SCC clinical trials. As part of that responsibility, the DSMC reviews all local serious adverse events in real time as they are reported and reviews adverse events on a quarterly basis. The quality assurance activity for the Clinical Research Office provides for periodic auditing of clinical research documents to ensure data integrity and regulatory compliance.

The SCC DSMC meets quarterly and conducts annual comprehensive reviews of ongoing clinical trials, for which it serves as the DSMC of record.

11.4 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study subject requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

11.4.1 Emergency Modifications

Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB approval.

For any such emergency modification implemented, an IRB modification form must be completed within five (5) business days of making the change.

11.4.2 Other Protocol Deviations/Violations

All other planned deviations from the protocol must have prior approval by the Principal Investigator and the IRB. According to the IRB, a protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs
- Has no substantive effect on the risks to research participants
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected
- Did not result from willful or knowing misconduct on the part of the investigator(s).

An unplanned protocol variance is considered a violation if the variance:

- Has harmed or increased the risk of harm to one or more research participants.
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

11.5 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator. When an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required.

The written amendment, and if required the amended consent form, must be sent to the IRB for approval prior to implementation.

11.6 Record Retention

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that the study investigator retain all study documentation pertaining to the conduct of a clinical trial. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

11.7 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms. Periodically, monitoring visits may be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

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