A phase II study assessing the safety and efficacy of oral testosterone undecanoate followed by enzalutamide as therapy for men with metastatic castrate resistant prostate cancer (CRPC)

The AcroBAT Study

IND#: 157109-Exempt

Principal Investigator
Samuel Denmeade, M.D.

Johns Hopkins University
GU Prostate Cancer Program
Skip Viragh Bldg – Room 9121
201 N. Broadway
Baltimore, MD 21287

Tel 410-955-8875
Fax 410-367-2668

email: denmesa@jhmi.edu

Statistician
Hao Wang, Ph.D.

Division of Biostatistics & Bioinformatics,
SKCCC at Johns Hopkins
JHU School of Medicine, Baltimore, MD
550 North Broadway, Room 1101
Baltimore, MD 21205

Tel 410-614-3426

email: hwang76@jhmi.edu

Co-Principal Investigator
Shifeng S. Mao, M.D.

Allegheny General Hospital
Allegheny-Singer Research Institute
320 East North Avenue
Pittsburgh, PA 15212

Tel 412-359-8373
Fax 412-442-2198

email: Shifeng.mao@ahn.org

Project Manager
Amber Michalik, BA

Johns Hopkins University
GU Prostate Cancer Program
Skip Viragh Bldg 9th floor
201 N. Broadway
Baltimore, MD 21287

Tel 410-502-0756
Fax 410-367-2668

email: amichal2@jhmi.edu

Lead Research Nurse Manager
Rana Sullivan, RN
GU Prostate Cancer Program
Skip Viragh Bldg – Room 9133
201 N. Broadway
Baltimore, MD 21287

Tele 410-614-6337
Fax 410-367-2668

email: tomalra@jhmi.edu

Participating Sites:
Johns Hopkins University
Allegheny General Hospital

SYNOPSIS:

Title: A phase II study assessing the safety and efficacy of oral testosterone undecanoate followed by enzalutamide as therapy for men with metastatic castrate resistant prostate cancer (mCRPC)

Objectives: The primary objective is to determine the objective response rate (ORR) of men with newly castration resistant metastatic prostate cancer following administration of oral testosterone undecanoate (OT).

Study Design: Single-arm, two-site, open label study of the effects of OT followed by enzalutamide in men with metastatic CRPC who previously progressed on therapy for metastatic castration-sensitive prostate cancer (CSPC) and have measurable disease.

Treatment Plan: Thirty patients will be enrolled. Enrolled patients will have baseline PSA and testosterone measured and obtain bone/CT scans. They will continue on androgen ablative therapy with LHRH agonist (i.e. Zoladex, Trelstar, Eligard or Lupron) or LHRH antagonist (Degarelix, Relugolix) if not surgically castrated to suppress endogenous testosterone production. Patients will receive OT at the maximum dose of 396 mg (two 198 mg capsules) twice per day on days 1-7 and 15-21 of a 28-day cycle. Testosterone and PSA levels will be measured 2 hours after the first dose (Cycle D1) and within 24-96 hours after Cycle 1 D7 (i.e. between Day 8-10) to determine peak and trough testosterone levels and stimulation of PSA production. Every 3 cycles (i.e. ~ 3 months), patients will have repeat PSA and bone/CT scans to establish the effect of OT on these parameters. Following radiographic progression on OT and a wash-out period of 21 days, patients will receive enzalutamide 160 mg daily for three 28-day cycles followed by PSA and a CT scan and bone scan to establish the response rate to enzalutamide at 3 months following OT. Patients with objective progression will come off study. Patients with stable disease or objective response will remain on enzalutamide for an additional 3 cycles and then be reassessed. Patients showing objective progression at this point will come off study.

Patients with continued PSA or objective response will come off study but continue on enzalutamide as standard of care therapy.

Assessment of Response to OT (To occur after every 3 cycles):

1) Patients without evidence of clinical or radiographic progression will continue OT until evidence of clinical or radiographic progression.
2) Patients with evidence of clinical or radiographic progression on CT scan (per RECIST criteria) will stop OT and will proceed to treatment with enzalutamide.
3) Patients with radiographic progression on bone scan (per PCWG3 criteria as ≥ 2 new bone lesions) will stop OT and proceed to therapy with enzalutamide, except for the first reassessment scan. If radiographic progression is detected on the first bone scan after starting OT (but not the CT scan), then the patient will remain on OT and have a confirmatory scan performed 12 weeks (3 cycles later). If this confirmatory scan shows 2 or more additional new lesions, this defines progression. The date of radiographic progression is the date of the first assessment bone scan. If the confirmatory scan does not show any additional new lesions, the patient will remain on OT. If progression is observed on subsequent bone scans, a confirmatory scan is not required; the date of this bone scan is the date of progression and the patient will stop OT and proceed to therapy with enzalutamide.

Assessment of Response to enzalutamide post-OT (To occur after 3 cycles):

At time of progression on OT, patients will remain on LHRH agonist alone for 21 days to re-establish a castrate level of T (<50 ng/dl). At this point they will begin treatment with enzalutamide at a dose of 160 mg p.o. per day. Cycles will be 28 days. PSA response will be assessed every cycle. Objective response will be assessed by repeat CT scan after 3 and 6 cycles.

1) Radiographic response on CT scan will be defined per RECIST criteria for patients with measurable disease.

2) Patients will come off study after radiographic or clinical progression or after completion of 6 cycles of enzalutamide with decision to continue or stop enzalutamide as standard of care therapy at the discretion of the patient's primary oncologist.

Study Population: Men with asymptomatic metastatic CRPC with rising PSA who have been treated with continuous castrating androgen deprivation therapy. Patients must have measurable disease by RECIST 1.1. Specifically, patients must have at least one measurable lesion ≥10mm with CT scan. Patients must not have received prior treatment for CRPC. As treatment for CSPC, patients are permitted to have received ADT alone, ADT and bicalutamide, ADT and docetaxel, ADT and abiraterone. Specifically, patients are not permitted to have received prior enzalutamide, apalutamide, or darolutamide.

Number of Patients: 30

Inclusion criteria:

1. Performance status ≤2
2. Age ≥18 years
3. Histologically-confirmed adenocarcinoma of the prostate
4. Evidence of metastatic, measurable disease by CT scan. Measurable disease is defined by RECIST 1.1 as at least one measurable lesion ≥10mm by CT scan.
5. Progressing on first line therapy with either continuous androgen deprivation therapy (ADT) (either surgical castration or LHRH agonist/antagonist) alone, or ADT in combination with bicalutamide or next generation androgen receptor inhibitors (abiraterone, enzalutamide, darolutamide).
6. Documented castrate level of serum testosterone (<50 ng/dl)
7. Patients progressing on combination therapy will continue ADT but must be off anti-androgen or abiraterone for 4 weeks prior to first treatment with OT.
8. Patients receiving prednisone in conjunction with abiraterone acetate must be

weaned off prednisone if possible prior to starting OT. Patients who cannot be weaned entirely off prednisone must be maintained on lowest stable dose that relieves symptoms.

9. Rising PSA on two successive measurements at least two weeks apart. Screening PSA must be ≥ 1.0 ng/mL.
10. Patients must continue on castrating therapy throughout OT treatment.
11. Prior docetaxel for CSPC is permitted if ≤ 6 doses were given in conjunction with first-line androgen deprivation therapy and >6 months since last dose of docetaxel.
12. Acceptable liver function:
    a. Bilirubin < 2.5 times institutional upper limit of normal (ULN)
    b. AST (SGOT) and ALT (SGPT) < 2.5 times ULN
13. Acceptable renal function:
    a. Serum creatinine < 2.5 times ULN, OR
14. Acceptable hematologic status:
    a. Absolute neutrophil count (ANC) ≥ 1500 cells/mm^3 (1.5×10^9/L)
    b. Platelet count ≥ 100,000 platelet/mm^3 (100×10^9/L)
    c. Hemoglobin ≥ 9 g/dL.
15. At least 4 weeks since prior surgery with full recovery (no persistent toxicity ≥ Grade 1).
16. Ability to understand and willingness to sign a written informed consent document.

Exclusion criteria:

1. Pain due to metastatic prostate cancer requiring opioid analgesics.
2. Prior treatment with any agent (such as docetaxel, cabazitaxel, anti-androgen, abiraterone, or investigational agent) for metastatic castration-resistant prostate cancer is prohibited.
3. Requires urinary catheterization for voiding due to obstruction secondary to prostatic enlargement thought to be due to prostate cancer or benign prostatic

hyperplasia. Patients with indwelling catheter/suprapubic catheter to relieve obstruction are eligible.

4. Evidence of disease in sites or extent that, in the opinion of the investigator, would put the patient at risk from therapy with testosterone (e.g. femoral metastases with concern over fracture risk, epidural spinal metastases with concern over spinal cord compression, lymph node disease with concern for ureteral obstruction).
5. Evidence of serious and/or unstable pre-existing medical, psychiatric or other condition (including laboratory abnormalities) that could interfere with patient safety or provision of informed consent to participate in this study.
6. Active uncontrolled infection, such as HIV/AIDS or chronic hepatitis B or untreated chronic hepatitis C.
7. Prior history of a thromboembolic event within the past two years and not currently on systemic anticoagulation.
8. Uncontrolled hypertension defined as sustained blood pressure (BP) >150 mm Hg systolic or > 90 mm Hg diastolic despite optimal antihypertensive treatment
9. Stroke (including transient ischemic attack), myocardial infarction, or other symptomatic ischemic event
10. Significant cardiovascular disease (such as New York Heart Association Class II or greater cardiac disease, unstable arrhythmia, or unstable angina) within 6 months prior to initiation of study treatment
11. History of clinically significant ventricular dysrhythmias or risk factors for ventricular dysrhythmias such as structural heart disease (e.g., severe left ventricular systolic dysfunction, left ventricular hypertrophy), coronary heart disease (symptomatic or with ischemia demonstrated by diagnostic testing)
12. Patients on Coumadin must be switched to alternative anticoagulation) Patients receiving anticoagulation therapy with warfarin, rivaroxaban or apixaban are not eligible for study. [Patients on enoxaparin or edoxaban are eligible for study. Patients on warfarin, rivaroxaban or apixaban, who can be transitioned to enoxaparin prior to starting study treatments, will be eligible

13. Hematocrit >50%, untreated severe obstructive sleep apnea, uncontrolled or poorly controlled heart failure [per Endocrine Society Clinical Practice Guidelines].

Study Endpoints:

Primary:

1. Objective response rate (ORR) on OT (defined by RECIST 1.1 and PCWG3)

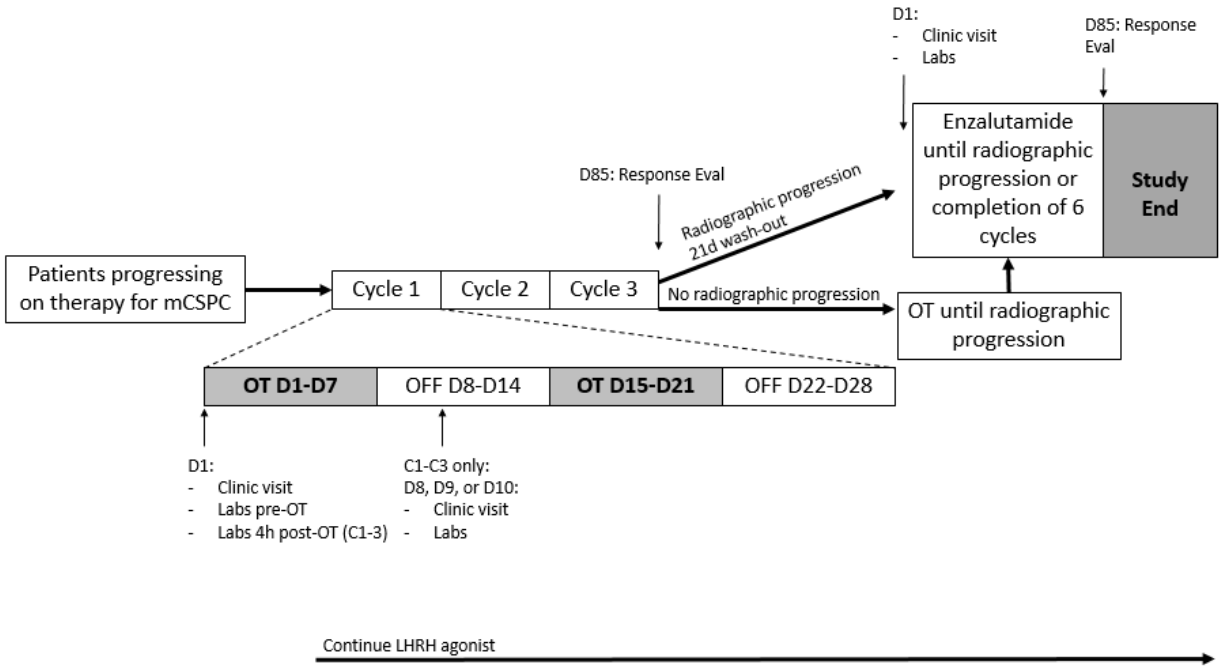
Secondary:

1. PSA response to OT (≥50% PSA reduction from baseline PSA level)
2. Time to PSA progression on OT
3. Time to clinical or radiographic progression on OT (defined by RECIST 1.1 and PCWG3)
4. PSA response to enzalutamide (≥50% PSA reduction from pre-enzalutamide PSA level)
5. ORR on enzalutamide (defined by RECIST 1.1 and PCWG3)
6. Peak and trough testosterone levels on OT
7. Quality of life metrics (FACIT-Fatigue Scale, SF-36)
8. Phenotype of PBMCs
9. Safety and Tolerability
    a. Adverse events will be collected at each clinic visit and classified for severity according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.03. Determination of relationship of the adverse event to the study procedures or study drug will be performed by the PI using the standard attribution terminology (e.g. unrelated, possibly related, etc.).

Exploratory Endpoints:

1. Time to PSA progression on enzalutamide from start of enzalutamide
2. Time to PSA progression on enzalutamide from start of OT (PSA-PFS2)

Study Scheme:



Statistical Plan:

The primary endpoint is the ORR to OT. A sample size of 30 patients will give 90% power to reject the null ORR of 5% and detect an ORR of 25% (5 or more ORs) with one-sided target type I error of 0.05.

TABLE OF CONTENTS

Page

SYNOPSIS **2**

STUDY SCHEMA **7**

TABLE OF CONTENTS… **8**

1. INTRODUCTION **10**

1.1 Testosterone as treatment for prostate cancer 10

1.2 Clinical experience with bipolar androgen therapy (BAT) 10

1.3 Rationale for testing of oral testosterone 13

2. STUDY OBJECTIVES **13**

2.1 Primary Objectives 28

2.2 Secondary Objectives 29

3. PATIENT POPULATION AND SELECTION **29**

3.1 Inclusion Criteria 28

3.2 Exclusion Criteria 30

3.3 Inclusion of Minorities and Women 31

4. TREATMENT PLAN **32**

4.1 Study Design 32

4.2 Treatment Scheme 33

4.3 Dosing Delay and Modifications 32

4.4 Removal of Patients from Study 32

4.5 Criteria for Discontinuation… 34

4.6 Pain Management 35

5. TREATMENT ASSESSMENTS **35**

5.1 Screening Studies 36

5.2 Treatment Period 36

5.3 Early Discontinuation 37

6. STUDY ASSESSMENTS **38**

6.1 Assessing PSA Response to BAT 38
6.2 Assessing Response PSA response post-BAT 39
6.3 Assessing Response in Measurable Disease 40
6.4 Bone scan to quantify bone uptake of tracer 42
6.5 Safety Assessment 42
7. PHARMACEUTICAL INFORMATION-Testosterone undecanoate 43
8. STUDY CALENDAR 48
9. DATA REPORTING and REGULATORY REQUIREMENTS 50
9.1 Data Entry
9.2 Case Report Forms Completion
9.3 Source Documents
9.4 Record Retention
9.5 Data Management
9.6 Study Monitoring and Quality Assurance
9.7 Data and Safety Monitoring
10. ADVERSE EVENT MONITORING 50
10.1 Evaluating Adverse Events 51
10.2 Serious Adverse Events 52
10.3 Expedited Reporting 53
10.4 Institutional Review Board (IRB) Reporting at Participating Sites…….
10.5 Protocol Amendments 53
10.6 Informed Consent 54
11. STATISTICAL METHODS 54
12. CORRELATIVE STUDIES… x
13. REFERENCES 58
14. APPENDICES 62

1. INTRODUCTION

1.1 Testosterone as treatment for prostate cancer

Metastatic prostate cancer is a highly significant disease that claims the lives of ~30,000 American men each year. Androgen deprivation therapy (ADT) is initially very effective but is never curative as all men eventually develop castrate resistant prostate cancer (CRPC). A major factor driving resistance is the ability of prostate cancer (PCa) cells to adapt to the chronic low androgen conditions by upregulating androgen receptor (AR) activity through overexpression, gene amplification and expression of truncated, transcriptionally active AR variants that lack the ligand-binding domain. Persistent signaling through AR makes CRPC sensitive to more potent inhibition of AR by abiraterone acetate or second generation anti-androgens such as enzalutamide. Yet these therapies have a limited duration of benefit prior to development of resistance, often through further increase in AR levels.

While this marked upregulation of AR can drive resistance, we have demonstrated that it also creates a therapeutic vulnerability to exposure to high levels of androgen. This was first demonstrated in preclinical models of CRPC in which high doses of androgen were shown to inhibit growth of CRPC cell lines and mouse xenografts. Multiple mechanisms likely contribute to this effect, including inhibition of c-Myc, induction of dsDNA breaks, AR licensing of cell cycle progression, and induction of ferroptosis. However we have also found that sustained exposure of CRPC to supraphysiological levels of androgens results in downregulation of AR and acquired resistance to this therapy. Therefore we have developed a therapy called Bipolar Androgen Therapy (BAT) in which testosterone-cypionate 400 mg IM is administered every 28 days to result in cycling from supraphysiological (>1500 ng/dL) to near-castrate levels. The rationale for cycling was that high serum T would kill high AR expressing CRPC while low serum T would prevent adaptation to high T and kill low AR expressing CRPC.

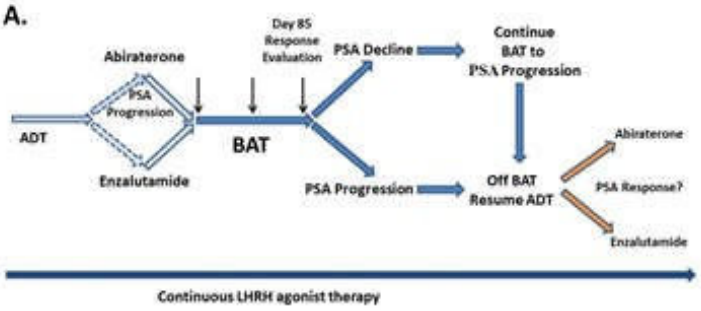
1.2 Clinical experience with BAT

To date we have treated ~250 men with BAT across four completed studies in asymptomatic men with CPRC. The key findings have been that BAT: (a) could be safely administered; (b) did not produce symptomatic disease progression; (c) produced sustained PSA and objective responses in ~30-40% of patients; (d) re-sensitized and prolonged response of patients to subsequent antiandrogen therapy. While ADT for advanced PCa often produces debilitating sexual and metabolic side effects, another highly significant feature of this approach is that BAT can make men feel remarkably better by decreasing fatigue, increasing physical activity and restoring libido and sexual function. BAT also produced favorable effects on body composition by increasing skeletal muscle mass and decreasing subcutaneous and visceral fat. Thus, incorporation of BAT into the treatment paradigm has the potential to improve the quality of life and well-being of PCa patients and minimize the morbidity from the metabolic sequelae produced by androgen ablative therapies.

RE-sensitizing with Supraphysiologic T to Overcome Resistance (RESTORE) Study: The RESTORE study (NCT02090114) was an NIH sponsored Phase II study designed to assess the safety and efficacy of BAT in men with CRPC progressing on either Enza (Cohort A) or Abi (Cohort B), (n=30 per cohort) (Figure 1A)[1]. The primary endpoint was the PSA response to BAT. Secondary endpoints included objective response, safety, and quality of life. Both cohorts met the primary endpoint. PSA50 Response to BAT was 30% in patients post-Enza and 18% post-Abi (Figure 1B)[1].

The second part of the study was to determine whether BAT could re-sensitize patients to repeat exposure to second line therapy that patients were progressing on prior to receiving BAT. Thus, patients progressing on Enza were re-exposed to Enza after BAT and similarly for Abi. Remarkably, the PSA50 response to re-exposure to Enza was 71%, although it was only 21% for patients re-exposed to Abi, (Figure 1B). Adverse events to

A.



B.

BAT Pre-Treatment	Total	PSA50	%	95% CI[1]
Two cohorts of the trial				
Last Drug Enza (Cohort A)	30	9	30.0%	[16.3%, 47.6%]
Last Drug Abi (Cohort B)	28	5	17.9%	[7.3%, 35.7%]
Post-hoc analysis				
Combined Cohort A and B	58	14	24.1%	[13.9%, 36.6%]
Two drugs (Abi-Enza or Enza-Abi)	21	6	28.6%	[13.2%, 50.6%]
One Drug (Abi or Enza)	37	8	21.6%	[9.8%, 38.2%]
Post-BAT Rechallenge Response				
Enza	21	15	71.0%	
Abi	19	4	21.0%	
Combined Cohorts A and B	40	19	48.0%	

[1]Both Cohorts A and B successfully met the endpoint for PSA50 Response
[2]Any PSA Response denotes any decline in PSA within first 3 cycles of BAT

C.

Any Grade 1-2 AE occurring on BAT in ≥10% of subjects	Frequency
Musculoskeletal Pain	40%
Increased Hemoglobin	37%
Anemia	17%
Breast Tenderness	17%
Rash	17%
Fatigue	17%
Nausea	17%
Gynecomastia	13%
Hot Flashes	13%
Hypertension	13%
Pruritis	13%
Abdominal pain	10%
Edema	10%

D.

Domain	p value
SF-36 Instrument	
-Physical Function	0.0468
-Emotional Well Being	0.0197
-Energy-Fatigue	0.0025
-General Health	0.8736
-Pain	0.6526
-Emotional Limits	0.5824
-Physical Health Limits	0.0152
-Social Functioning	0.2735
PANAS-SF Screen Positive Response	0.4977
PANAS-SF Screen Negative Response	0.7756
FACIT Fatigue Scale	0.0026
IIEF	1.7e-08

Paired Wilcoxon test for each QoL metric.
P values were not adjusted for multiple comparison.

Figure 1. Clinical data from the RESTORE Study. (A) Trial design; (B) Response parameters for patients progressing on Enza (Cohort A) or Abi (Cohort B). PSA50 response to rechallenge post-BAT with Enza was 71% vs 21% for Abi. (C) Adverse events due to BAT in at least 10% of patients on study. (D) Results of QOL instruments comparing 12 weeks treatment with BAT vs. baseline on ADT alone.

BAT were primarily Grade 1-2 with most common being generalized musculoskeletal pain, breast tenderness, hot flashes and gynecomastia (Figure 1C). Serious adverse events occurred in individual patients and were not attributed to BAT with exception of grade 3 hypertension that occurred in three patients. QoL instruments showed significant improvement in Physical Function, Emotional Well-Being and Energy-Fatigue on the SF-36 Instrument subscales, improvement in the FACIT Fatigue Scale and improvement in the IIEF survey for patients receiving BAT compared to baseline on ADT, (Figure 1D).

The RESTORE study also had a cohort of patients (N=29) who had not been treated with prior therapy for CRPC (cohort C). These patients exhibited a PSA50 response rate of 14% and rPFS of 8.5 months for patients with metastatic disease[2]. Most notably, 18 patients went on to receive AR ablative therapy with abiraterone or enzalutamide after progression on BAT, and the PSA50 response rate was found to be 94% and the PSA-PFS not reached with 11.2 months of follow-up for these patients. This suggested that BAT may prime CRPC to respond to subsequent AR ablative therapy.

The TRANSFORMER Study: The TRANSFORMER study (NCT02286921) was a DOD-sponsored randomized Phase II study designed to compare the efficacy of BAT vs. Enza in asymptomatic men with CRPC progressing on Abi, Figure 2A. The study was conducted at 17 academic centers across the US. The study was coordinated by the study team at Johns Hopkins who performed regulatory oversight, study monitoring, payment of invoices and data analyses in conjunction with the CRO Cancer Research and Biostatistics (CRAB) based in Seattle, WA. From 2015-2018, 195 patients underwent 1:1 randomization to either standard dose Enza (n=101) or BAT (n=94) at 400 mg IM every 28 days. The primary endpoint was clinical/radiographic progression free survival. At time of progression, patients were given the option to crossover to the alternate therapy. The trial was designed to show a 50% improvement in the primary endpoint for BAT vs. Enza. The trial did not meet the primary endpoint with PFS of 5.62 m for the BAT arm and 5.72 m for the Enza arm (p=0.2267), figure 2B. Objective response was 24.4% for BAT and 4.2% for Enza (p=0.067). Best PSA50 response was 28.4% for BAT and 25.5% for Enza (p=0.697). Overall BAT was well tolerated compared to Enza. BAT also improved quality of life compared to Enza particularly in the areas of fatigue, physical and sexual functioning.

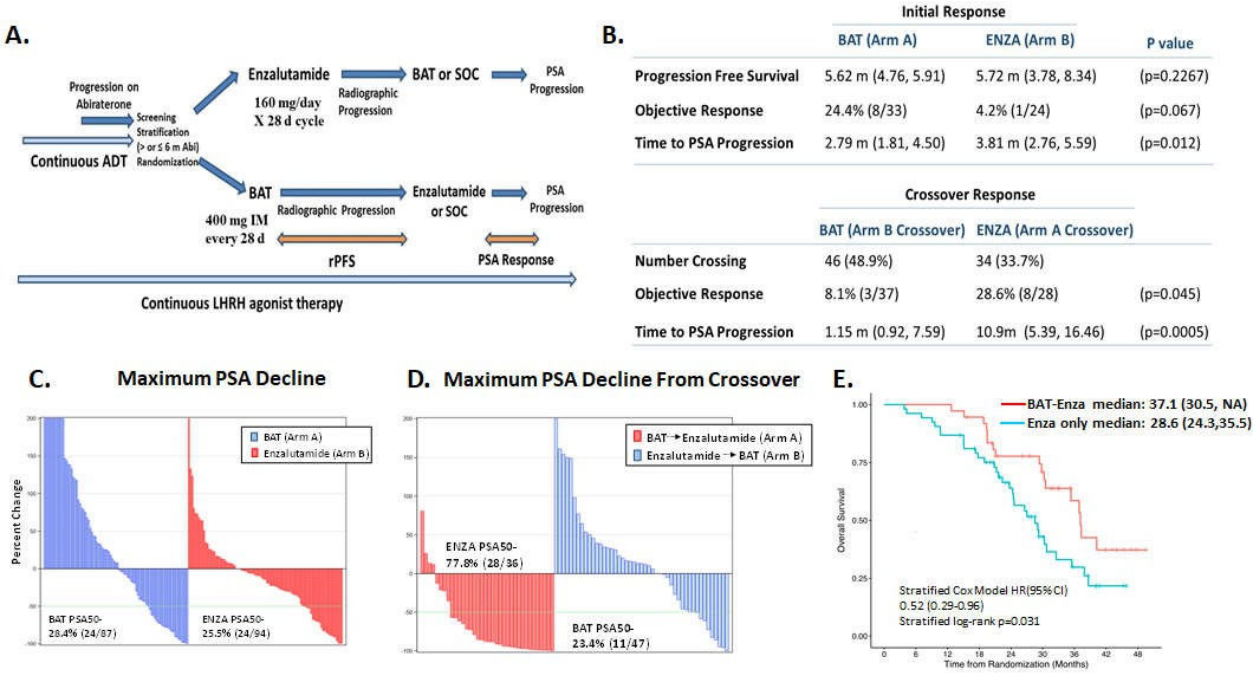


Figure 2. Clinical data from the TRANSFORMER Study. (A) Trial design; (B) Response parameters for BAT (Arm A) vs. Enza (Arm B) and results from patients who crossed over to opposite treatment. (C) Waterfall plots of PSA response showing PSA50 of 28.4% for BAT and 25.5% for Enza. (D) Waterfall plot of PSA50 response at crossover 77.8% response in patients crossing from BAT to Enza and 23.4% for patients crossing from Enza to BAT. (E) Kaplan-Meier curve of overall survival demonstrates significant increase in survival for BAT-Enza (37.1 months) vs. patients receiving Enza alone without BAT (28.6 months) (HR=0.52, p=0.031).

At time of progression, 47 (49%) of patients on the Enza arm crossed over to receive BAT and 36 (33.7%) crossed over from BAT to receive Enza and were available for analysis. Remarkably, the PSA50 response for patients who received Enza after BAT was 77.8% compared to 25.5% for patients receiving Enza immediately after Abi, (Figure 2C-D). Objective response in the Enza post-BAT patients was 28.6% compared to 4.2% post-Abi (Figure 2B). Time to PSA progression on Enza was also markedly improved, increasing almost 3-fold from 3.8 months post-Abi to 10.9 months post-BAT (Figure 2B). Post-hoc analysis of PSA progression to first and second stage of the study (PSA PFS2) for all patients revealed a median of 14.2 months for the sequence of Enza crossing over to BAT but 28.2 months for sequence of BAT crossing over to Enza. Overall survival for patients receiving BAT and then Enza was 37.1 months compared to only 28.6 months for those receiving Enza alone (HR 0.52,p=0.031), (Figure 2E).

1.3 Rationale for testing of oral testosterone

The studies performed to date demonstrate the safety and efficacy of high dose T in men with metastatic CRPC who are progressing on androgen ablative therapy. Our limited

data suggests that high serum levels of T may be required for clinical response. This high level is achieved through intramuscular (IM) administration of testosterone cypionate at the highest FDA-approved dose of 400 mg every 28 days. This regimen initially produces high serum T levels but these levels drop to a varying degrees approaching near castrate levels in some men over a 28-day cycle. To date, we have not tested whether more rapid cycling of serum T would produce similar or improved therapeutic response in this patient population. Additional issues with IM testosterone are (1) it requires patients to come to hospital every 28 days for injection, (2) has highly variable pharmacokinetics, (3) can cause physical discomfort from IM injections.

Recently two novel oral testosterone (OT) agents were developed for the treatment of male hypogonadism. Historically, an oral option for testosterone replacement therapy was unavailable because of risks of liver toxicity, including cholestasis and jaundice, associated with earlier developed 17-alpha-alkylated oral analogs. These novel oral agents feature specialized formulations that avoid adverse hepatic effects. In clinical trials, both oral agents produced no significant adverse effects on liver function tests. We have formed a collaborative partnership with Clarus Therapeutics, the makers of Jatenzo, an oral lipoprotein-coated testosterone undecanoate (OT) formulation that was FDA-approved in December 2019 as T-replacement therapy and commercially available in 2020. Published pharmacokinetic data demonstrate that OT can produce supraphysiologic serum T levels when administered at the highest FDA-approved dose. Therefore, our hypothesis is that OT, given at the highest FDA approved dose, will achieve more homogeneous and sustained supraphysiologic levels of serum T that will produce similar or enhanced therapeutic response. To avoid adaptation of CRPC to this sustained level of T, we will utilize a dosing schedule of 1 week-on, 1 week-off.

2. STUDY OBJECTIVES

2.1 Primary Objective

1. The primary objective is to determine the objective response rate (ORR) of men with newly castration resistant metastatic prostate cancer following administration of oral testosterone undecanoate (OT).

2.2. Secondary Objectives

1. PSA response to OT (≥50% PSA reduction from baseline PSA level)
2. Time to PSA progression on OT

3. Time to clinical or radiographic progression on OT (defined by RECIST 1.1 and PCWG3)
4. PSA response to enzalutamide (≥50% PSA reduction from pre-enzalutamide PSA level)
5. ORR on enzalutamide (defined by RECIST 1.1 and PCWG3)
6. Peak and trough testosterone levels on OT
7. Quality of life metrics (FACIT-Fatigue, RANDSF-36)
8. Phenotype of PBMCs
9. Safety and Tolerability - Adverse events will be collected at each clinic visit and classified for severity according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.0. Determination of relationship of the adverse event to the study procedures or study drug will be performed by the PI using the standard attribution terminology (e.g. unrelated, possibly related, etc.).
10. Time to PSA progression on enzalutamide from start of enzalutamide
11. Time to PSA progression on enzalutamide from start of OT (PSA-PFS2)

3. PATIENT POPULATION AND SELECTION

Men with asymptomatic metastatic CRPC with rising PSA who have been treated with continuous castrating androgen ablative therapy. Patients must have measurable disease by RECIST 1.1. Specifically, patients must have at least one measurable lesion ≥10mm with CT scan. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should have been confirmed by histology. Patients must not have received prior treatment for CRPC. As treatment for CSPC, patients are permitted to have received ADT alone, ADT and bicalutamide, ADT and docetaxel, ADT and abiraterone. Specifically, patients are not permitted to have received prior enzalutamide, apalutamide, or darolutamide.

3.1 Inclusion Criteria

1. Performance status ≤2
2. Age ≥18 years
3. Histologically-confirmed adenocarcinoma of the prostate
4. Evidence of metastatic, measurable disease by CT scan. Measurable disease is defined by RECIST 1.1 as at least one measurable lesion ≥10mm by CT scan.
5. Progressing on first line therapy with either continuous androgen deprivation therapy (ADT) (either surgical castration or LHRH agonist/antagonist) alone, or ADT in combination with bicalutamide or next generation androgen receptor inhibitors (abiraterone, enzalutamide, darolutamide).
6. Documented castrate level of serum testosterone (<50 ng/dl)
7. Patients progressing on combination therapy will continue ADT but must be off anti-androgen or abiraterone for 4 weeks prior to first treatment with OT.
8. Patients receiving prednisone in conjunction with abiraterone acetate must be weaned off prednisone if possible prior to starting OT. Patients who cannot be weaned entirely off prednisone must be maintained on lowest stable dose that relieves symptoms.
9. Rising PSA on two successive measurements at least two weeks apart. Screening PSA must be ≥ 1.0 ng/mL.
10. Patients must continue on castrating therapy throughout OT treatment.
11. Prior docetaxel for CSPC is permitted if ≤ 6 doses were given in conjunction with first-line androgen deprivation therapy and >6 months since last dose of docetaxel.
12. Acceptable liver function:
    a. Bilirubin < 2.5 times institutional upper limit of normal (ULN)
    b. AST (SGOT) and ALT (SGPT) < 2.5 times ULN
13. Acceptable renal function:
    a. Serum creatinine < 2.5 times ULN, OR
14. Acceptable hematologic status:
    a. Absolute neutrophil count (ANC) ≥ 1500 cells/mm^3 (1.5×10^9/L)
    b. Platelet count ≥ 100,000 platelet/mm^3 (100×10^9/L)

c. Hemoglobin ≥ 9 g/dL.

15. At least 4 weeks since prior surgery with full recovery (no persistent toxicity ≥ Grade 1).
16. Ability to understand and willingness to sign a written informed consent document.

3.2 Exclusion criteria

1. Pain due to metastatic prostate cancer requiring opioid analgesics.
2. Prior treatment with any agent (such as docetaxel, cabazitaxel, anti-androgen, abiraterone, or investigational agent) for metastatic castration-resistant prostate cancer is prohibited.
3. Requires urinary catheterization for voiding due to obstruction secondary to prostatic enlargement thought to be due to prostate cancer or benign prostatic hyperplasia. Patients with indwelling catheter/suprapubic catheter to relieve obstruction are eligible.
4. Evidence of disease in sites or extent that, in the opinion of the investigator, would put the patient at risk from therapy with testosterone (e.g. femoral metastases with concern over fracture risk, epidural spinal metastases with concern over spinal cord compression, lymph node disease with concern for ureteral obstruction).
5. Evidence of serious and/or unstable pre-existing medical, psychiatric or other condition (including laboratory abnormalities) that could interfere with patient safety or provision of informed consent to participate in this study.
6. Active uncontrolled infection, such as HIV/AIDS or chronic hepatitis B or untreated chronic hepatitis C.
7. Prior history of a thromboembolic event within the past two years and not currently on systemic anticoagulation.
8. Uncontrolled hypertension defined as sustained blood pressure (BP) >150 mm Hg systolic or > 90 mm Hg diastolic despite optimal antihypertensive treatment

9. Stroke (including transient ischemic attack), myocardial infarction, or other symptomatic ischemic event
10. Significant cardiovascular disease (such as New York Heart Association Class II or greater cardiac disease, unstable arrhythmia, or unstable angina) within 6 months prior to initiation of study treatment
11. History of clinically significant ventricular dysrhythmias or risk factors for ventricular dysrhythmias such as structural heart disease (e.g., severe left ventricular systolic dysfunction, left ventricular hypertrophy), coronary heart disease (symptomatic or with ischemia demonstrated by diagnostic testing)
12. Patients on Coumadin must be switched to alternative anticoagulation. Patients receiving anticoagulation therapy with warfarin, rivaroxaban or apixaban are not eligible for study. [Patients on enoxaparin or edoxaban are eligible for study. Patients on warfarin, rivaroxaban or apixaban, who can be transitioned to enoxaparin prior to starting study treatments, will be eligible
13. Hematocrit >50%, untreated severe obstructive sleep apnea, uncontrolled or poorly controlled heart failure [per Endocrine Society Clinical Practice Guidelines].

3.3 Inclusion of women and minorities

This study is focused on prostate cancer, therefore is applicable to men only. Women will not be included on this study. Members from all ethnic and race groups are eligible for this study.

4. TREATMENT PLAN

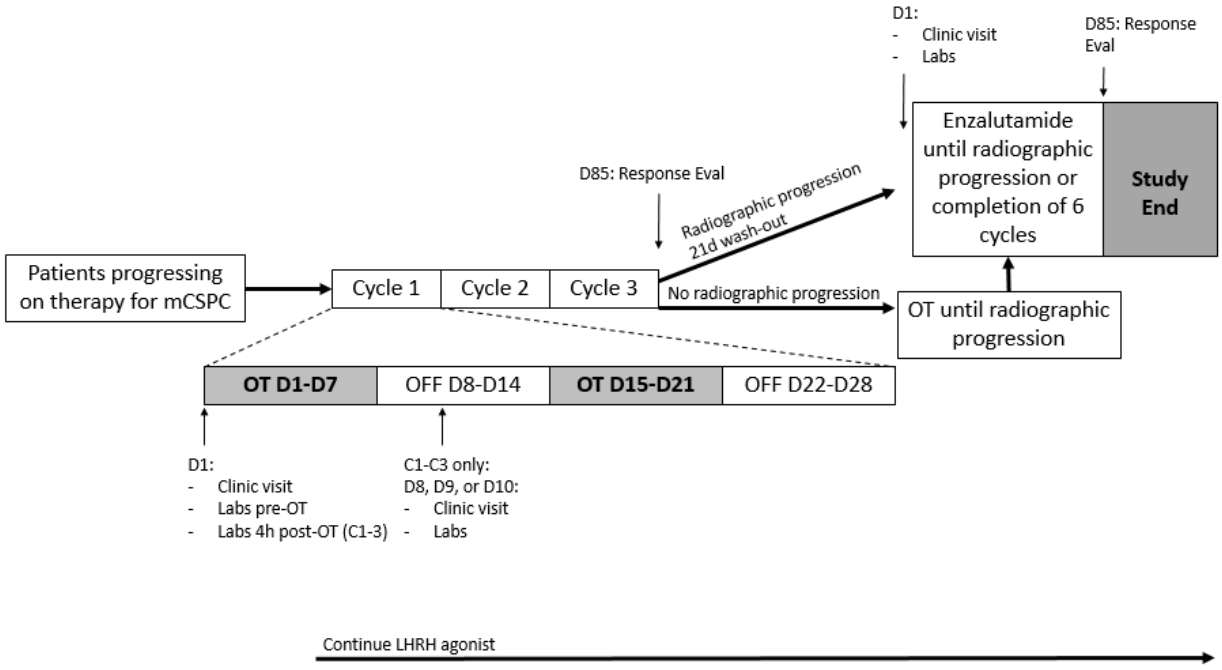
4.1 Study Design

This is a single-arm, two-site, open label study of the effects of OT followed by enzalutamide in men with metastatic CRPC who previously progressed on therapy for metastatic castration-sensitive prostate cancer (CSPC) and have measurable disease.

Treatment Plan: Thirty patients will be enrolled. Enrolled patients will have baseline PSA and testosterone measured and obtain bone/CT scans. They will continue on

androgen ablative therapy with LHRH agonist (i.e. Zoladex, Trelstar, Eligard or Lupron) if not surgically castrated to suppress endogenous testosterone production. Patients will receive OT at the maximum dose of 396 mg (two 198 mg capsules) twice per day on days 1-7 and 15-21 of a 28 day cycle. Oral testosterone will be provided to patient by the study. Testosterone and PSA levels will be measured 2 hours after the first dose and within 24-96 hours after Cycle 1 D7 (i.e. between Day 8-10) to determine peak and trough testosterone levels and stimulation of PSA production. Every 3 cycles (i.e. ~ 3 months), patients will have repeat PSA and bone/CT scans to establish the effect of OT on these parameters. Following radiographic progression on OT and a wash-out period of 21 days, patients will receive enzalutamide 160 mg daily and followed by PSA and a CT scan and bone scan to establish the response rate to enzalutamide over a 6 month period following OT. Enzalutamide will not be provided by the study and must be obtained from local pharmacy. PSA response will be assessed every cycle. Objective response will be assessed by repeat CT scan and bone scan after 3 and 6 cycles. Radiographic response on CT scan will be defined per RECIST criteria for patients with measurable disease. Patients will come off study after radiographic or clinical progression or after completion of 6 cycles of enzalutamide with decision to continue or stop enzalutamide as standard of care therapy at the discretion of the patient's primary oncologist.

4.2 Treatment Scheme:



4.3 Dosing Delays and Modifications

Treatment will be given on indicated days ± 2 days.

4.3.1 Testosterone Undecanoate Dose Modification for Elevated Blood Pressure

Table 1 provides treatment guidelines for hypertension deemed related to testosterone undecanoate. Blood pressure (BP) should be monitored in a constant position visit to visit, either sitting or supine in a relaxed setting. Decisions to reduce or interrupt the dose of study treatment must be based on BP readings taken by a medical professional and must be confirmed with a second measurement at least 5 minutes following the first measurement.

Testosterone Undecanoate (Jatenzo) should be discontinued in patients with hypertensive emergency.

Table 1 Management of Hypertension Associated with Testosterone Undecanoate (Jatenzo)

Criteria for Dose Modifications	Testosterone Undecanoate Dose Modification
≥ 160 mm Hg (systolic) OR ≥ 90 mm Hg (diastolic)	• Patient should be referred to Primary Care Physician to discuss addition of new or additional antihypertensive medications and/or increase dose of existing medications with the. Monitor patient closely for hypotension. • If optimized antihypertensive therapy (usually to include 3 agents) does not result in BP < 150 mm Hg systolic and < 90mm Hg diastolic, Testosterone Undecanoate treatment should be dose reduced by 20% • Testosterone Undecanoate treatment should be dose interrupted for up to 7 days if upper limits of systolic BP (≥ 160 mmHg) are sustained and not adequately manageable or if systolic BP is > 180 mmHg or sustained diastolic BP > 110 mm Hg, or if patient is symptomatic • If BP remains above upper limits of systolic (≥ 160 mmHg) or diastolic (>90 mm Hg) after 20% dose reduction, patient should discontinue testosterone undecanoate.
Hypertensive emergency[a]	• Discontinue testosterone undecanoate treatment

[a]Hypertensive emergency is defined as uncontrolled elevated BP with clinical evidence of progressive or impending end-organ damage (e.g., myocardial infarction/ischemia, intracranial hemorrhage, cerebral ischemia, pulmonary edema, encephalopathy, kidney damage).

4.4 Removal of Patients from Study

A patient may be removed from the study for a variety of reasons, including:

1. Clinical or radiographic evidence of progression
2. Unacceptable adverse event(s)

- Patients develop uncontrolled hypertension with sustained elevated BP (systolic >160; diastolic >90 mm Hg) despite optimized antihypertensive regimen and 20% dose reduction of testosterone undecanoate
- Patients develop urinary outlet obstruction thought to be due to prostate cancer within the prostate and requiring urinary catheterization
- Patients who develop grade 3 or higher liver function abnormalities with increase in bilirubin, AST (SGOT) or ALT (SGPT) ≥ 2.5 times institutional upper limit of normal (ULN)

- Patients develop decreased renal function with serum creatinine ≥ 2.5 times baseline level
- Patents develop hypersensitivity or anaphylactoid reactions to oral testosterone .
- Patients develop nausea/vomiting that cannot be controlled with oral antiemetic regimen.
- Intercurrent illness that prevents further participation

3. Experiencing a treatment delay of longer than 2 weeks due to drug toxicity; however, if the patient is receiving clinical benefit, treatment may be delayed for longer than 2 weeks and then resumed at the discretion of the Investigator.
4. Patient refuses further treatment through the study and/or withdraws consent to participate
5. Patients is noncompliant with respect to taking drugs, keeping appointments, or having tests required for the evaluation of drug safety and efficacy
6. General or specific changes in the patient's condition that render the patient unacceptable for further treatment in this study in the judgment of the investigator.
7. Under no circumstance will care of a withdrawn patient be adversely affected by a decision to withdraw or be withdrawn from the study.

4.5 Criteria for Discontinuation of Study Treatment due to Development of Worsening Pain

Due to the nature of the treatment, there is concern that patients could experience potential development of prostate cancer related pain due to OT. This worsening of pain was not observed in any of the three studies that previously tested the use of testosterone as therapy for men with asymptomatic CRPC. In addition, men with baseline pain due to prostate cancer are excluded from enrolling in the study.

The primary efficacy endpoint of the study is objective response. However, if patients have unequivocal clinical progression without radiographic progression, these patients are indicated for the current standard of care. Study treatment should be stopped and patients advised regarding available treatment options. For this study, unequivocal clinical progression will be characterized as:

1. Cancer pain requiring initiation of chronic administration of opiate analgesia (oral opiate use for ≥3 weeks; parenteral opiate use for ≥7 days. Patients with cancer pain requiring opiate analgesia for relief should also be assessed by the investigator for the need for initiating systemic chemotherapy. Or
2. Immediate need to initiate cytotoxic chemotherapy or the immediate need to have either radiation therapy or surgical intervention for complications due to tumor progression, even in the absence of radiographic evidence of disease progression. Or
3. Deterioration in ECOG performance status to grade 3 or higher. Patients whose ECOG performance status decreases to grade 2 during the study should be assessed carefully for their need for alternate therapy.

4.6 Pain management:

Patients who develop new pain thought by the investigator to be due to prostate cancer should be treated according to NCCN guidelines for adult Cancer Pain (Ver.

2.2013) in a stepwise fashion using the WHO Analgesic Ladder approach. Patients with pain that is managed without oral opioids after 3 weeks can continue on to the next cycle of the study. Patients with a continued requirement for oral opioids after 3 weeks will be removed from study. Patients who have recurrent pain requiring oral opioids on subsequent cycles will also be removed from study. Patients with sustained or escalating pain after 3 weeks may be treated with daily bicalutamide at a dose of 150 mg at the investigator's discretion.

5. TREATMENT ASSESSMENT AND EVALUATION

1. Screening Studies are performed within 28 days before Cycle 1 Day 1. All required treatment and post-treatment study procedures and assessments must be done within 5 days (+/-) of the specified study visit date. Treatment will be given on indicated days ± 2 days.

5.1 Screening Studies (performed within 28 days before enrollment)

1. Comprehensive medical history, vitals and physical exam, including height and weight, and medications.
2. ECOG Performance status (PS)
3. CBC (Complete blood count) with differential and platelet count
4. CMP (Comprehensive Metabolic Panel - Sodium, Potassium, Chloride, BUN, Serum Creatinine, Calcium, Total Protein, Albumin, Total Bilirubin, AST, ALT, Alkaline Phosphatase, Bicarbonate)
5. Serum PSA, testosterone
6. Lipid Panel (fasting) (Total Cholesterol, HDL, LDL, Triglycerides)
7. Androgen Panel [dihydrotestosterone (DHT), free testosterone, estradiol, dehydroepiandostenedione sulfate (DHEA-S), DHEA
8. Baseline 12-lead, supine EKG

9. Partial Prothrombin (PTT) and Prothrombin Times (PT)
10. Staging imaging with CT (within 8 weeks before enrollment)
11. Staging imaging with bone scintigraphy (standard technetium-99m-MDP bone scan) (within 8 weeks before enrollment)
12. Quality of life surveys (FACIT-Fatigue Scale, RANDSF-36)

5.2 Treatment Period

1. Clinic visits every cycle for assessment of toxicity, ECOG performance status, vital signs and body weight.
2. D1 of cycles 1-3: **Pre-OT testosterone and PSA**, 2h **post-OT testosterone and PSA** (Cycle1-3 only)
3. Cycle1-3 only: D8, D9, or D10: testosterone and PSA
4. **CBC, Comp Panel** (Every other cycle)
5. **Lipid Panel** (fasting) Total Cholesterol, HDL, LDL, Tryglycerides (every 3 months times 3 while on OT treatment and every 3 months times 2 while on enza treatment)
6. **Androgen Panel-** [dihydrotestosterone (DHT), free testosterone, estradiol, dehydroepiandostenedione sulfate (DHEA-S), DHEA (every 3 months times 3 while on OT treatment and every 3 months times 2 while on enza treatment)
7. **PBMCs and Plasma-** Hopkins/Sibley Patients Only: (D1 each OT cycle, C1 D8 of OT cycle, OT reassessment visit and Enza reassessment visit)
8. **Standard CT scan** and conventional planar **whole-body bone scan** (i.e. standard bone scan) every 3 months

9. **Quality of life surveys** (FACIT-Fatigue Scale, RANDSF-36)- At baseline and after 3 months of OT

5.3 Early Discontinuation (To occur 28 days after early removal from the study)

1. Clinic visit
2. If early discontinuation is due to an adverse event or toxicity of any of the study treatments the patient should be followed until resolution of the adverse event/toxicity or at least one month, whichever is later.

6. STUDY ASSESSMENTS

6.1 Assessing Response in Measurable Disease (all patients)

Tumor response will be evaluated using CT and bone scan. Patients will undergo screening CT scan and bone scan every 3 months to determine disease response/progression to either BAT or Enzalutamide. Progression for soft tissue lesions will be based on RECIST 1.1 criteria and for bone lesions based on PCWG3 criteria.

<u>Assessing Radiographic Response to BAT:</u>

Patients with radiographic progression on CT scan (per RECIST criteria) will stop BAT regardless of PSA response and will proceed with enzalutamide.

Radiographic progression on bone scan will be defined by PCWG3 criteria as ≥ 2 new bone lesions. However, for the first assessment scan only, patients with bone scan progression should remain on study and have a confirmatory scan performed 12 weeks (3 cycles) later (Appendix 3). If this confirmatory scan shows 2 or more additional new lesions, this defines progression. The date of radiographic progression is the date of the first reassessment bone scan. If the confirmatory scan does not show any additional new lesions, patient remains on study. If progression is observed on subsequent bone scans, a confirmatory scan is not required; the date of this bone scan is the date of progression.

Patients with bone scan progression based on these criteria will stop BAT regardless of PSA response and will proceed with enzalutamide therapy.

Assessing Radiographic Response to Enzalutamide or Abiraterone post-BAT:

Patients with radiographic progression on CT scan (per RECIST criteria) will stop enzalutamide regardless of PSA response and will come off study.

Radiographic progression on bone scan will be defined by PCWG3 criteria as ≥ 2 new bone lesions. Patients with bone scan progression based on these criteria will stop enzalutamide.

6.2 Safety Assessment

Safety will be evaluated based on the incidence, severity, duration, causality, seriousness, and type of adverse events (AEs), and changes in the patient's physical examination, vital signs, and clinical laboratory results. Investigators will use the NCI CTCAE version 4.03 (published14 June 2010) to assess the severity of AEs and toxicities (see Appendix II).

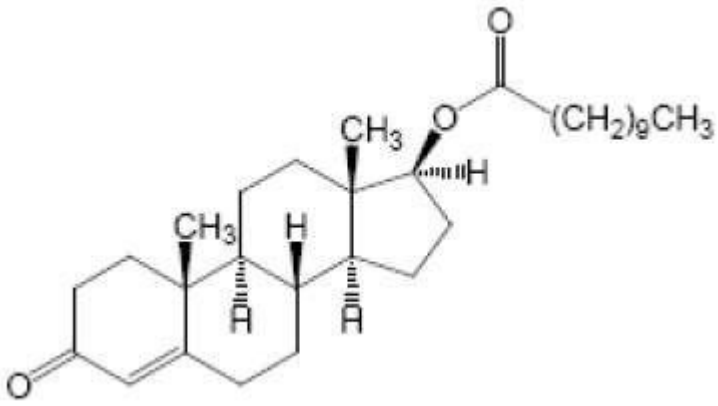
6.3 Assessing Response in PSA

PSA progression will be assessed using the PCWG3 criteria defined as: An increase in PSA greater than 25% and >2 ng/ml above nadir, confirmed by progression at 2 timepoints at least 3 weeks apart

7 PHARMACEUTICAL INFORMATION-Testosterone Undecanoate

7.1 Testosterone Undecanoate Drug Characterization

7.1.1 Drug Name: Testosterone Undecanoate (JATENZO)



7.1.2 Chemical Name: 17β-hydroxyandrost-4-en-3-one Undecanoate

7.1.3 Molecular Formula: C30H48O3 **Molecular Weight:** 456.7 g/mol

7.1.4 Solubility: Insoluble in water

7.1.5 Description

JATENZO for oral use is provided as a gelatin capsule containing testosterone undecanoate, a fatty-acid ester of testosterone. Testosterone undecanoate is a white to off-white yellow crystalline powder. Testosterone, an androgen, is formed by cleavage of the ester side chain of testosterone undecanoate.

JATENZO capsules are available in three strengths of 158 mg, 198 mg, and 237 mg. The 158 mg strength is an opaque red capsule that contains 158 mg of testosterone undecanoate and is imprinted with “158” in white ink. The 198 mg strength is an opaque white capsule that contains 198 mg of testosterone undecanoate and is imprinted with “198” in red ink. The 237 mg strength is an opaque orange capsule that contains 237 mg of testosterone undecanoate and is imprinted with “237” in white ink. All capsule strengths also contain oleic acid, polyoxyl 40 hydrogenated castor oil (Cremophor RH 40), borage seed oil, peppermint oil, and butylated hydroxytoluene as inactive ingredients. Gelatin capsule shells are composed of the following inactive ingredients: Gelatin, sorbitol, glycerin, purified water, iron oxide red, FD&C Yellow #6, and titanium dioxide.

7.2 Precautions

7.2.1 General

1. Patients with benign prostatic hypertrophy may develop acute urethral obstruction. Priapism or excessive sexual stimulation may develop.
2. Oligospermia may occur after prolonged administration or excessive dosage. If any of these effects appear, the androgen should be stopped and if restarted, a lower dosage should be utilized.

7.2.2 Information for patients

Patients should be instructed to report any of the following: nausea, vomiting, changes in skin color, ankle swelling, too frequent or persistent erections of the penis.

7.2.3 Laboratory tests

1. Hemoglobin and hematocrit levels (to detect polycythemia) should be checked periodically in patients receiving long-term androgen administration.
2. Serum cholesterol may increase during androgen therapy.

7.2.4 Drug interactions

1. Androgens may increase sensitivity to oral anticoagulants. Dosage of the anticoagulant may require reduction in order to maintain satisfactory therapeutic hypoprothrombinemia.
2. Concurrent administration of oxyphenbutazone and androgens may result in elevated serum levels of oxyphenbutazone.
3. In diabetic patients, the metabolic effects of androgens may decrease blood glucose and, therefore, insulin requirements.

7.2.5 Drug/Laboratory test interferences

Androgens may decrease levels of thyroxine-binding globulin, resulting in decreased total T4 serum levels and increased resin uptake of T3 and T4. Free thyroid hormone

levels remain unchanged, however, and there is no clinical evidence of thyroid dysfunction.

7.3 Adverse Reactions

The following adverse reactions in the male have occurred with some androgens:

1. Cardiovascular: Elevated Blood Pressure
2. Endocrine and urogenital: Gynecomastia and excessive frequency and duration of penile erections (priapism). Oligospermia may occur at high dosages.
3. Skin and appendages: Hirsutism, male pattern of baldness, seborrhea, and acne.
4. Fluid and electrolyte disturbances: Retention of sodium, chloride, water, potassium, calcium, and inorganic phosphates.
5. Gastrointestinal: Nausea, cholestatic jaundice, alterations in liver function tests, rarely hepatocellular neoplasms and peliosis hepatitis.
6. Hematologic: Suppression of clotting factors II, V, VII, and X, bleeding in patients on concomitant anticoagulant therapy, polycythemia, thrombosis.
7. Nervous system: Increased or decreased libido, headache, anxiety, depression, and generalized paresthesia.
8. Allergic: Hypersensitivity, including skin manifestations and anaphylactoid reactions.

7.3.1 Drug Abuse and Dependence

Controlled Substance Class:

JATENZO contains testosterone undecanoate, which is a Schedule III controlled substance as defined under the Controlled Substances Act.

7.3.2 Overdosage

There have been no reports of acute overdosage with the androgens.

7.4 Administration, Supply and Storage

7.4.1 Administration:

JATENZO is an oral medication that should be taken with food. The dosing schedule in this study is 396 mg by mouth twice daily on days 1-7 and 15-21 of a 28 day cycle. The capsules are not to be crushed or chewed, must be swallowed whole. If the dose is vomited, do not replace, start with the next scheduled dose. If the dose is missed for more than 2 hours, resume normal dosing schedule

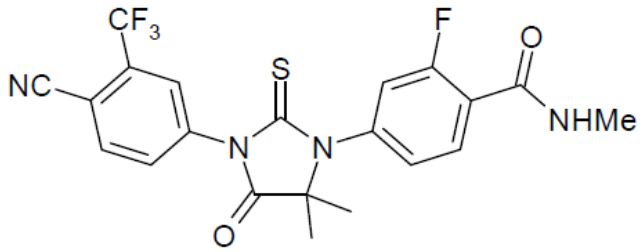
7.4.2 Supply

JATENZO (testosterone undecanoate) capsules are available in three strengths of 158 mg, 198 mg, and 237 mg. Capsules are packaged as 120 units in wide-mouth, round, white HDPE bottles with white, polypropylene, child resistant caps and induction-sealed liner. 158 mg capsules are opaque red capsules imprinted with “158” in white ink and are supplied in bottles: NDC 69087-158-12.
198 mg capsules are opaque white capsules imprinted with “198” in red ink and are supplied in bottles: NDC 69087-198-12.
237 mg capsules are opaque orange capsules imprinted with “237” in white ink and are supplied in bottles: NDC 69087-237-12.

7.4.3 Storage:

Store at 20°C to 25ºC (68°F to 77ºF), excursions permitted to 15ºC to 30ºC (59ºF to 86ºF). Avoid exposing the capsules to moisture (store in a dry place).

9.7 Enzalutamide Drug Characterization [Adapted from FDA prescribing information]



- **Chemical Name:** 4-{3-[4-cyano-3-(trifluoromethyl)phenyl]-5,5dimethyl-4-oxo-2-sulfanylideneimidazolidin-1-yl}-2-fluoro-*N*-methylbenzamide
- **Molecular Formula:** $C_{21}H_{16}F_4N_4O_2S$ **Molecular Weight:** 464.44 g/mol
- **Description:** Enzalutamide is a white crystalline non-hygroscopic solid. It is practically insoluble in water. Enzalutamide is provided as liquid-filled soft gelatin capsules for oral administration. Each capsule contains 40 mg of enzalutamide as a solution in caprylocaproyl polyoxylglycerides. The inactive ingredients are caprylocaproyl polyoxylglycerides, butylated hydroxyanisole, butylated hydroxytoluene, gelatin, sorbitol sorbitan solution, glycerin, purified water, titanium dioxide, and black iron oxide.

Clinical Pharmacology

Following oral administration of enzalutamide 160 mg daily (the current FDA approved dose) in patients with metastatic castration-resistant prostate cancer, the median time to reach maximum plasma enzalutamide concentrations (Cmax) is 1 hour (range 0.5 to 3 hours). At steady state, the plasma mean Cmax values for enzalutamide and N-desmethyl enzalutamide (enzalutamide's major active metabolit) are 16.6 µg/mL (23% CV) and 12.7 µg/mL (30% CV), respectively, and the plasma mean predose trough values are 11.4 µg/mL (26% CV) and 13.0 µg/mL (30% CV), respectively. The mean apparent volume of distribution (V/F) of enzalutamide in patients after a single oral dose is 110 L (29% CV).

Enzalutamide is 97% to 98% bound to plasma proteins, primarily albumin. N-desmethyl enzalutamide is 95% bound to plasma proteins. The mean apparent clearance (CL/F) of enzalutamide in patients after a single oral dose is 0.56 L/h (range 0.33 to 1.02 L/h). The mean terminal half-life (t1/2) for enzalutamide in patients after a single oral dose is 5.8 days (range 2.8 to 10.2 days). Following a single 160 mg oral dose of enzalutamide in healthy volunteers, the mean terminal t1/2 for N-desmethyl enzalutamide is approximately 7.8 to 8.6 days. In healthy volunteers, a high-fat meal did not alter the AUC to enzalutamide or N-desmethyl enzalutamide.

In vitro, human CYP2C8 and CYP3A4 are responsible for the metabolism of enzalutamide. Based on *in vivo* and *in vitro* data, CYP2C8 is primarily responsible for the

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

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

Safety/Precautions

- **Seizures:** In the Phase III post-docetaxel randomized clinical trial, 7 of 800 (0.9%) patients treated with enzalutamide 160 mg once daily experienced a seizure. No seizures occurred in patients treated with placebo. Patients experiencing seizure were permanently discontinued from therapy and all seizures resolved. There is no clinical trial experience re-administering enzalutamide to patients who experienced seizures.

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

  Because of the risk of seizure associated with XTANDI use, patients should be advised of the risk of engaging in any activity where sudden loss of consciousness could cause serious harm to themselves or others.
- **Laboratory abnormalities:** In the randomized Phase III post-docetaxel trial, Grade 1-4 neutropenia occurred in 15% of patients on enzalutamide (1% Grade 3-4) and in 6% of patients on placebo (no Grade 3-4). The incidence of Grade 1-4 thrombocytopenia was similar in both arms; 0.5% of patients on enzalutamide and 1% on placebo experienced Grade 3-4 thrombocytopenia. Grade 1-4 elevations in ALT occurred in 10% of patients

on enzalutamide (0.3% Grade 3-4) and 18% of patients on placebo (0.5% Grade 3-4). Grade 1-4 elevations in bilirubin occurred in 3% of patients on enzalutamide and 2% of patients on placebo.

- **Infections:** In the randomized Phase III post-docetaxel trial, 1.0% of patients treated with enzalutamide compared to 0.3% of patients on placebo died from infections or sepsis. Infection-related serious adverse events were reported in approximately 6% of the patients on both treatment arms.
- **Falls and fall-related injuries:** In the randomized Phase III post-docetaxel trial, falls or injuries related to falls occurred in 4.6% of patients treated with enzalutamide compared to 1.3% of patients on placebo. Falls were not associated with loss of consciousness or seizure. Fall-related injuries were more severe in patients treated with enzalutamide and included non-pathologic fractures, joint injuries, and hematomas.
- **Hallucinations:** In the randomized Phase III post-docetaxel trial, 1.6% of patients treated with enzalutamide were reported to have Grade 1 or 2 hallucinations compared to 0.3% of patients on placebo. Of the patients with hallucinations, the majority were on opioid-containing medications at the time of the event. Hallucinations were visual, tactile, or undefined.
- **Unforeseeable risks to embryo or fetus:** Enzalutamide is contraindicated in women. In theory, enzalutamide can cause fetal harm if administered to a pregnant woman based on its mechanism of action. While there are no human or animal data on the use of enzalutamide in pregnancy and enzalutamide is not indicated for use in women, it is important to know that maternal use of an androgen receptor inhibitor could affect development of the fetus. Women who are pregnant or women who may be pregnant should not handle enzalutamide without protection, e.g., gloves. Patients should also be informed that it is not known whether enzalutamide or its metabolites are present in semen and they should use a condom if having sex with a pregnant woman. The patient should use a condom and another effective method of birth control if he is having sex with a woman of child-bearing potential. These measures are required during and for three months after treatment with enzalutamide.

Information for Patients

- Instruct patients to take their dose at the same time each day (once daily). enzalutamide can be taken with or without food. Each capsule should be swallowed whole. Do not

chew, dissolve, or open the capsules. If the dose is vomited, do not replace, start with the next scheduled dose.

- Inform patients that enzalutamide has been associated with an increased risk of seizure. Discuss conditions that may predispose to seizures and medications that may lower the seizure threshold. Advise patients of the risk of engaging in any activity where sudden loss of consciousness could cause serious harm to themselves or others.
- Inform patients that enzalutamide may cause dizziness, mental impairment, paresthesia, hypoesthesia, and falls.
- Inform patients that if they miss a dose, then they should take it as soon as they remember. If they forget to take the dose for the whole day, then they should take their normal dose the next day. They should not take more than 160 mg of enzalutamide per day.
- Apprise patients of the common side effects associated with enzalutamide. Direct the patient to a complete list of adverse drug reactions in the FDA-approved patient labeling (PATIENT INFORMATION).
- Inform patients that enzalutamide may be harmful to a developing fetus. Patients should also be informed that they should use a condom if having sex with a pregnant woman. A condom and another effective method of birth control should be used if the patient is having sex with a woman of child-bearing potential. These measures are required during and for three months after treatment with enzalutamide.

Laboratory tests

- Liver function tests (e.g. AST/ALT, bilirubin) should be monitored periodically while on enzalutamide.
- Complete blood counts (e.g. white blood cell count, hemoglobin, hematocrit and platelet count) should be monitored periodically while on enzalutamide.

Drug Interactions

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. Co-administration of enzalutamide with strong CYP2C8 inhibitors should be avoided if possible. If co-administration of

enzalutamide with a strong CYP2C8 inhibitor cannot be avoided, reduce the dose of enzalutamide.

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

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

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

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

For more detail on specific drug interactions, please refer to the FDA prescribing information.

Adverse Reactions

The most common adverse drug reactions (≥ 5%) reported in patients receiving enzalutamide in the randomized clinical trial were asthenia/fatigue, back pain, diarrhea, arthralgia, hot flush, peripheral edema, musculoskeletal pain, headache, upper respiratory infection, muscular weakness, dizziness, insomnia, lower respiratory infection, spinal cord compression and cauda equina syndrome, hematuria, paresthesia, anxiety, and hypertension. Grade 3 and higher adverse reactions were reported among 47% of enzalutamide -treated patients and 53% of placebo-treated patients. Discontinuations due to adverse events were reported for 16% of enzalutamide -treated patients and 18% of placebo-treated patients. The most common adverse reaction leading to treatment discontinuation was seizure, which occurred in 0.9% of the enzalutamide -treated patients compared to none (0%) of the placebo-treated patients. Table 2 summarizes the adverse reactions reported in the randomized Phase II clinical trials.

Administration, Supply and Storage

Enzalutamide is marketed but not approved for the indication under study.

Administration

Enzalutamide 160 mg daily (the current FDA approved dose), or four 40 mg capsules by mouth daily, will be administered.

Supply

Enzalutamide, marketed as Xtandi, comes in 40 mg capsules and are supplied as white to off-white oblong soft gelatin capsules imprinted in black ink with MDV. Enzalutamide capsules are available in bottles of 120 capsules (NDC 0469-0125-99). Drug will be obtained through approval from the patient's private medical insurance and supplied by their local pharmacy.

Storage

Store enzalutamide capsules at 20°C to 25°C (68°F to 77°F) in a dry place and keep the container tightly closed. Excursions permitted from 15°C to 30°C (59°F to 86°F).

9. STUDY CALENDAR

	Study Period																	
	Screening	On Testosterone Therapy (OT on D1-7 and D15-21 of a 28 day cycle)											On Enzalutamide Tx (28 day cycles)					Off Tx
	Day -D28 to D1	C1D1 PreTx V1	C1D1 2h post +/- 30 min V1	C1 D8, 9, or 10[1] V2	C2D1 PreTx V3	C2D1 2h post +/- 30 min V3	C2 D8, 9, or 10[1] V4	C3D1 PreTx V5	C3D1 2h post +/- 30 min V5	C3 D8, 9, or 10[1] V6	Response [2a,3] Reassessment V7	Extended OT Cycles [2b,9] Every28 Days	C1D1 Enza V1	C2D1 Enza V2	C3D1 Enza V3	Response[4,5] Reassessment Enza V4	Extended Enza TX 3 Cycles max[4]	

Informed Consent	X																	
Complete Medical H&P	X																	
Vital Signs (BP, HR, RR, T)	X	X			X			X			X	X	X	X	X	X	X	X
Height (screening only) Weight with BMI	X	X			X			X			X	X	X	X	X	X	X	X
Clinic Visit	X	X		X	X		X	X		X	X	X	X	X	X	X	X	X
ECOG PS	X	X			X			X			X	X	X	X	X	X	X	X
Concomitant Medications	X	X			X			X			X	X	X	X	X	X	X	X
ECG	X																	
PT/PTT	X																	
PSA	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serum Testosterone	X	X	X	X	X	X	X	X	X	X	X	X	X					
CBC with differential[10]	X				X						X	X	X	X	X	X	X	X
Complete Metabolic Panel[10]	X				X						X	X	X	X	X	X	X	X
Androgen Panel[6]	X										X	X[6]	X			X		
Lipid Panel[6]	X										X	X[6]	X			X		
Bone Scan [7, 9]	X										X	X [2b]				X	X[4]	
Chest/Abdomen/Pelvis CT scan [7, 9]	X										X	X [2b]				X	X[4]	
Jatenzo(Oral Testosterone)		X			X			X				X						
Enzalutamide													X	X	X	X	X	
PBMC blood sample (JHH/Sibley only)		X		X	X			X			X					X		
Plasma sample (JHH/Sibley only)		X		X	X			X			X					X		
FACIT-F[8]	X										X							
RANDSF-36[8]	X										X							

[1]Blood draw for testosterone at 2 hr after 1st dose and between D8-10 is only required for first three cycles of OT.

[2a]On day 85 of the OT Treatment Period **(End of OT Cycle 3),** patients will be assessed for radiographic response.
[2b]Patients without radiographic progression will continue to receive OT and the OT Treatment Period schedule will be repeated until time of radiographic progression (reassessment CT and Bone Scan every 3 cycles).

[3]If patient has progression on day 85 of the OT Treatment Period **(End of OT Cycle 3),** they will not receive any therapy and will return in 21 days to start Enzalutamide.

[4]On day 85 of the Enza Treatment Period **(End of Enza Cycle 3),** patients will be assessed for radiographic response. Patients without radiographic progression will continue to receive Enza every 28 days and the Enza-only Treatment Period schedule will be repeated until time of radiographic progression or 6 months of treatment, whichever comes first. **Patients with continued PSA or objective response will come off study but continue on enzalutamide as standard of care therapy.**

[5]Patients with radiographic progression on day 85 of the Enza-only Treatment Period **(End of Enza Cycle 3)** will be removed from study.

[6]**Androgen panel** [dihydrotestosterone (DHT), free testosterone, estradiol, dehydroepiandostenedione sulfate (DHEA-S), DHEA will be done every 3 months x 3 total while on OT and every 3 months x 2 total while on Enzalutamide.
Lipid Panel (fasting) (Total Cholesterol, HDL, LDL, Triglycerides) will be done every 3 months x 3 total while on OT and every 3 months x 2 total while on Enzalutamide

[7]Baseline CT scan and bone scan will be allowed within 8 weeks before enrollment.

[8]QOL surveys will be done at baseline and after 3 cycles of OT.

[9]For patients who respond to OT for >12 months, it is left to treating physician's discretion to increase CT and bone scan interval to every 4-6 months.

[10]Blood draw for CBC with differential and Comprehensive Metabolic Panel-every other cycle (i.e., screening, cycle 2, cycle 4, etc)

9. DATA REPORTING AND REGULATORY REQUIREMENTS

Multicenter Guidelines

The Protocol Chair

Each institution will have a Protocol Chair (Dr. Samuel Denmeade and Dr. Shifeng Mao), who will be responsible for performing the following tasks:

- Coordinating, developing, submitting, and obtaining approval for the protocol as well as its subsequent amendments.
- Assuring that all participating institutions are using the correct version of the protocol.
- Taking responsibility for the overall conduct of the study at all participating institutions and for monitoring the progress of the study.
- Reviewing and ensuring reporting of Serious Adverse Events (SAEs).
- Reviewing data from all sites.

Each institution will be responsible for the following tasks:

- Ensuring that IRB approval has been obtained at each participating site prior to the first patient registration at that site, and maintaining copies of IRB approvals from each site.
- Managing central patient registration.
- Collecting and compiling data from each site.
- Establishing procedures for documentation, reporting and submitting of AEs and SAEs to the Protocol Chair and all other applicable parties.
- Facilitating audits by securing selected source documents and research records from participating sites for audit, or by auditing at participating sites.

All sites are responsible for performing the following tasks:

- Following the protocol as written, and the guidelines of Good Clinical Practice (GCP).
- Submitting data to the Lead Center.
- Registering all patients with the Lead Center by submitting patient registration form and signed informed consent promptly.
- Providing sufficient experienced clinical and administrative staff and adequate facilities and equipment to conduct a collaborative trial according to the protocol.
- Maintaining regulatory binders on site and providing copies of all required documents to the Lead Center.
- Collecting and submitting data according to the schedule specified by the protocol.

9.1 Data Entry

Data collected during this study will be entered into a secure database. Staff at Johns Hopkins University will be responsible for the initial study configuration and setup in the CRMS database and for any future changes.

9.1.1 Case Report Forms Completion

Electronic Case report forms will be generated by the coordinating center for the collection of all study data. Investigators will be responsible for ensuring that the CRFs are kept up-to-date.

The paper Eligibility Checklist CRF must be completed using black ink. Any errors must be crossed out so that the original entry is still visible, the correction clearly indicated and then initialed and dated by the individual making the correction.

eCRFs will be completed within 2 weeks of the patient coming to the clinic and all relevant supporting documentation such as scans, progress notes, nursing notes, blood work, pathology reports, etc., will be submitted to the Study Manager for remote monitoring approximately every 4-6 weeks when requested. All patient names or other identifying information will be removed prior to being sent to the Coordinating Center (SKCCC) or non-redacted source documents can be sent via a password-protected/secured document transfer based on each institution's guidelines.

9.1.2 Source Documents

Study personnel will record clinical data in each patient's source documents (i.e. the patient's medical record). Source documentation will be made available to support the patient research record. Study monitors will review entries on the CRFs at regular intervals, comparing the content with source documents.

9.1.3 Record Retention

The Investigator will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. After study closure, the Investigator will maintain all source documents, study- related documents, and the CRFs. Because the length of time required for retaining records depends upon a number of regulatory and legal factors, documents should be stored until the Investigator is notified that the documents

may be destroyed. In this study, records are to be retained and securely stored for a minimum of 5 years after the completion of all study activities.

9.2 Data Management

9.2.1 Lead Research Program Coordinators

A Lead research program coordinator at the leading institutions will be assigned to the study. A Lead Research Program Coordinator will manage the study activities at each of the participating sites. The responsibilities of the Lead Research Program Coordinator include project compliance, data collection, data entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordination of the activities of the protocol team.

9.3 Study Monitoring and Quality Assurance

Regularly scheduled registration reports will be generated to monitor patient accruals and the completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and the extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period, and potential problems will be brought to the attention of the principal investigator for discussion and action.

Random-sample data quality and protocol compliance audits may be conducted by the study team at least once a year, more frequently if indicated. Audits by the coordinating center may entail (1) submitting source documents and research records for selected patients from participating sites to the coordinating center for audit, or (2) on-site auditing of selected patient records at participating sites.

All clinical work conducted under this protocol is subject to Good Clinical Practice (GCP) guidelines. This includes inspection of study-related records by the lead site, sponsor, its designee, or health authority representatives at any time.

9.4 Data and Safety Monitoring

The SKCCC Compliance Monitoring Program will provide external monitoring for JHU-affiliated sites in accordance with SKCCC DSMP (Version 6.0, 02/21/2019). The SMC Subcommittee will

determine the level of patient safety risk and level/frequency of monitoring. The Clinical Research Office will perform an audit after the first subject has been treated and then periodically depending on the rate of accrual and prior audit results. All trial monitoring and reporting will be reviewed annually by the SKCCC Safety Monitoring Committee. The PI is responsible for internally monitoring the study. Data must be reviewed to assure the validity of data, as well as, the safety of the subjects. The PI will also monitor the progress of the trial, review safety reports, and clinical trial efficacy endpoints and to confirm that the safety outcomes favor continuation of the study.

Additionally, scheduled meetings will take place monthly and will include the protocol Principal Investigator, research nurse, data manager, and, when appropriate, the collaborators, subinvestigators, and biostatistician involved with the conduct of the protocol.

During these meetings the investigators will discuss matters related to: the safety of protocol participants, validity and integrity of the data, enrollment rate relative to expectation, characteristics of participants, retention of participants, adherence to protocol (potential or real protocol violations), data completeness, and progress of data for secondary objectives.

10 Adverse Event Monitoring and Reporting

An Adverse Event is defined as any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure (attribution of unrelated, unlikely, possible, probable, or definite). The PI and/or the research nurse will monitor each patient closely for the development of adverse events and toxicities and record all such events. Patients will be evaluated for toxicity if they have received one dose of testosterone undecanoate or one 28-day cycle of enzalutamide. The timely reporting of adverse events (including toxic deaths) is required by the Food and Drug Administration (FDA).

10.10 Evaluating Adverse Events

The grade and severity of the event will be determined using the DCT/NCI Common Terminology Criteria, CTCAE v.4.03. Links to CTCAE version 4.03 can be found in Appendix 2. All appropriate treatment areas should have access to a copy of the CTCAE version 4.03. Study staff must use one of the CTCAE criteria to define the event. Adverse events not

included in the CTCAE v.4.03 should be reported and graded under the “Other” adverse event within the appropriate category and grade 1 to 5 according to the general grade definitions, mild, moderate, severe, life-threatening, fatal or disabling, as provided in the CTCAE.

The event will be determined to be expected or unexpected. The determination of whether an AE is expected is based on agent-specific adverse event information provided in Section 9 Pharmaceutical Information. Unexpected AEs are those not listed in the agent-specific adverse event information provided in Section 9 Pharmaceutical Information.

The event will be evaluated for relationship to the medical treatment or procedure. The Investigator should document his/her opinion of the relationship of the event to study medication as follows:

□ *Unrelated*- The adverse event is clearly not related to the investigational agent(s).
□ *Unlikely*- The adverse event is doubtfully related to the investigational agent(s).
□ *Possible*-The adverse event may be related to the investigational agent(s).
□ *Probable*-The adverse event is most likely related to the investigational agent(s).
□ *Definite*- The adverse event is clearly related to the investigational agent(s).

Based on this information, a decision will be made whether an adverse event should be reported as an expedited report (Serious Adverse Event, section 3.0) in addition to the routinely reported clinical data. All expedited adverse event reports should be submitted to the JHM Institutional Review Board (IRB) and to the FDA.

10.11 Documenting Adverse Events

Each individual sign or symptom must be documented separately.. All adverse events (both expected and unexpected) will be captured on the appropriate study-specific case report forms (CRFs). CRFs must be signed and dated by person conducting evaluation to be used as source documentation

The attribution of all adverse events must be verified by an investigator. Evaluation of laboratory toxicities may be documented directly on a printed laboratory report or CRF provided it is signed by the investigator. However, if an action was conducted due to this abnormality (e.g. RBC transfusion due to low Hgb) this would be recorded on the AE

form also.

10.2 Serious Adverse Events

A SAE is any sign, symptom or medical condition that emerges during treatment or during a post-treatment follow-up period that (1) was not present at the start of treatment and is not a chronic condition that was part of the patient's medical history, OR (2) was present at the start of treatment or as part of the patient's medical history but worsened in severity and/or frequency during therapy, AND that meets any of the following regulatory criteria:

□ is fatal (i.e., results in death from any cause at any time) or life-threatening (i.e., the patient was in the view of the investigator, at immediate risk of death from the reaction as it occurred)
□ required or prolonged hospitalization (see exclusions below)
□ results in persistent or significant disability/incapacity
□ constitutes a congenital anomaly or a birth defect
□ is medically significant, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.
Events not considered to be serious adverse event are hospitalizations for the:
□ Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition
□ Treatment, which was elective or pre-planned, for a pre-existing condition that did not worsen
Any serious adverse event occurring in a patient from the first day of treatment and until 4 weeks after the last dose of treatment must be reported. The period after discontinuing study drug may be extended if there is a strong suspicion that the drug has not yet been eliminated.

All serious adverse events must be followed to resolution (≤ 1 or baseline) or until considered stable or irreversible.

10.3 Expedited Reporting of Serious Adverse Events

Serious adverse events and protocol problems will be reported in compliance with JHM IRB

guideline, “Organization Policy on Reports of Unanticipated Problems **Involving Risks to Participants or Others” [Policy No. 103.6(b)] (**most current version). A copy of this document is located at https://www.hopkinsmedicine.org/institutional_review_board/guidelines_policies/organization_policies/prompt_reporting_policy.html

All deaths on study regardless of attribution must be reported by the Principal Investigator Dr. Denmeade to the JHM IRB. In addition, all serious adverse events, regardless of causality to study drug and/or administration device, will be reported promptly to Dr. Denmeade (e-mail: denmesa@jhmi.edu) and to Lead Site Study Coordinator Serina King (email: sking18@jhmi.edu, (p) 410-614-6139, (f) 410-367-2668, within 24 hours of recognition of the serious adverse event. If this falls on a weekend or holiday, an email notification is acceptable but must be followed by an SAE reporting form on the next business day.

Investigators will be notified by the Principal Investigator and/or by the CRO of all SAEs that are unexpected (ie, not previously described in the Pharmaceutical Characterization of each drug in section 9 of this protocol), and definitely, probably, or possibly related to BAT or enzalutamide. This notification will be in the form of an expedited safety report (ESR) that is to be faxed to the investigators and the study coordinators within 48 hours. Upon receiving such notices, the site investigator must review and retain the notice and where required by local site regulations, the site investigator will submit the ESR to the site IRB. The site investigator and IRB will determine if the informed consent requires revision. The site investigator should also comply with the site IRB procedures for reporting any other safety information. Where required, submission of ESRs by the investigator to Health Authorities should be handled according to local regulations.

10.4 Institutional Review Board (IRB) Reporting at Participating Sites:

Participating sites will be responsible for reporting to their IRB. All serious adverse events will be reported to the IRB per institutional standards within 3 business days of recognition of the adverse event if the event is related and expected, related and unexpected, or related

and fatal or life-threatening due to administration of the investigational product. If the serious adverse event is unrelated to administration of the investigational agents, then it will be reported to the IRB within 15 days of recognition of the serious adverse event. Upon receipt of the report of the serious adverse event by IRB, follow-up information will be given to the IRB within 15 days.

10.5 Protocol Amendments:

Any changes to the protocol will be made in the form of an amendment and must be approved by the site IRB before implementation.

10.6 Informed consent:

Written informed consent will be obtained by a study investigator or study research nurse working on this study. An explanation of the nature of study, its purpose, procedures involved, expected duration, potential risks and benefits will be provided to each participant by the investigator or the research nurse. Each participant will be informed that participation in the study is voluntary and that he may withdraw from the study at any time, and that withdrawal of consent will not affect his subsequent medical treatment. Participants will be allowed time needed to make an informed decision. Participants will be encouraged to ask questions about the study and the consent before signing the consent form. Consent forms will be filed with the Clinical Research Office and copies stored securely with the study coordinator. No patient will enter the study before his informed consent has been obtained.

11. STATISTICAL METHODS

Study design and sample size determination: The primary endpoint is the ORR to OT. An objective response is defined per RECIST 1.1. A sample size of 30 patients will give 90% power to reject the null ORR of 5% and detect an ORR of 25% (5 or more ORs) with one-sided target type I error of 0.05. One final analysis is planned to determine ORR to OT.

The evaluable population for objective response includes the subjects who start OT and have at least one radiographic disease assessment after starting OT. The primary analysis will be

performed in the evaluable population. The analysis including all patients who receive at least one dose of OT will serve as supportive.

Analysis methods for efficacy endpoints: The primary endpoint is the ORR to OT. An objective response is defined per RECIST 1.1. The percentage of evaluable patients who exhibit an objective response of total evaluable patients will comprise the ORR.

The first secondary endpoint is PSA response rate to OT. PSA response is defined as a decrease in the PSA value (at any point during OT treatment) by 50% from the pre-treatment PSA determined on Cycle1D1. The percentage of patients who exhibit a PSA response among those who start OT and have at least one PSA measurement after starting OT will form the estimate of the PSA response rate. If a patient receives at least 3 cycles of OT but have missing PSA value will be treated as a non-responder and will be included in the denominator. We will also assess the time to PSA progression on OT. Time to PSA progression on OT is defined as the time from the date of initial dose of OT to the date of the PSA measurement when it shows an ≥25% increase above the nadir value and confirmed by a repeat PSA 4 weeks later (PCWG3 criteria) during the treatment with OT, and will be described using Kaplan-Meier method.

An additional secondary endpoint is time to clinical or radiographic progression on OT. Tumor response will be evaluated based on RECIST 1.1 for soft tissue lesions and on PCWG3 criteria for bone lesions. Time to clinical or radiographic progression is defined as the time from the date of initiation of OT to the date of new lesions on bone scan (per PCWG3 criteria) or progression on CT scan (per RECIST criteria) compared to baseline or clinical progression determined by the investigators. We will summarize time to clinical or radiographic progression using Kaplan-Meier method.

Additional secondary endpoints include PSA and OR rates to enzalutamide. PSA response is defined as a decrease in the PSA value (at any point during enza treatment) by 50% from the pre-treatment PSA determined on E1D1. An objective response is defined per RECIST 1.1. The percentage of evaluable patients who exhibit an objective response of total evaluable patients will comprise the ORR.

Additional secondary endpoints are the peak and trough testosterone levels on OT and phenotype of PBMCs. The peak testosterone level will be the average of the 4 hour post-treatment measurements determined on Cycle1D1, Cycle2D1, and Cycle3D1. The trough testosterone level will be the average of the measurements obtained between days 8-10 on cycles 1-3 of OT.

An exploratory endpoint will be the time to PSA progression on enzalutamide or post-OT, defined as the time from the date of initiation of enzalutamide to the date of the PSA measurement when it shows an increase by ≥25% above the nadir value that occurred following initiation of enzalutamide and confirmed by a repeat PSA 4 weeks later (PCWG3). We will summarize it using Kaplan-Meier method.

Analysis of quality of life, metabolic studies, and PBMC phenotyping: QoL will be assessed using RAND-SF36 Quality of Life Survey and FACIT-F Version 4. For each module, summary statistics of the scores will be reported at baseline and after 3 cycles of OT. Changes in quality of life scores pre- and post-treatment will be evaluated at each follow-up time by paired-sample t-tests or Wilcoxon signed rank tests as appropriate. In addition, mixture effect models will be fitted for accessing the quality of life changes over time. The change of metabolic measures (e.g., lipid profile, androgen panel, body composition, BMI) will be evaluated using the same analysis methods. The PBMC phenotype will be determined by multiplex flow cytometry. The change in PBMC phenotype will be evaluated by similar analysis methods.

Safety analysis: The overall safety profile and toleration of BAT and Enzalutamide, will be characterized by type, frequency, severity, timing and relationship of study therapy of adverse events and laboratory abnormalities. Adverse events will be summarized by the frequency of patients experiencing treatment emergent adverse events corresponding to body systems and by worst NCI CTCAE (version 4.03) grade.

12. Correlative Studies

1. **Peripheral blood mononuclear cell (PBMC) studies** – Hopkins/Sibley patients only

   Peripheral blood will be collected into **three (Brand: BD Vacutainer BD CPT) CPT tubes** according to the study calendar at day 1 each OT cycle, C1 D8 of OT cycle, OT reassessment visit and Enzalutamide reassessment visit. Following collection, Laura Sena from the Denmeade lab will be notified for specimen transfer (see contact information below). Sibley samples will be shipped to the Denmeade lab on same day as blood collection according to the instructions in the lab manual.

   Peripheral blood mononuclear cells (PBMCs) will be isolated and cryopreserved in freezing media (FBS, 10%DMSO). Cells will be stored long-term in liquid nitrogen until thawed for batch analysis. Upon thawing, cells will be immunophenotyped using multiparameter flow cytometry to evaluate the immunologic and metabolic phenotype of the following cell subsets: CD4 and CD8 T cells, FOXP3+ Tregs, B cells, NK cells, monocytes, dendritic cells, and myeloid derived suppressor cells (Table 1).

   Samples will be run on a Cytek Aurora machine, which allows for the simultaneous detection of up to 25 markers. Additionally, the metabolic fitness of the total PBMCs will be evaluated using the mitochondrial stress test on the Seahorse analyzer. Functional assays will be run on PBMCs to determine the relative capacity of peripheral T cells to metabolically respond to T cell receptor stimulation through activation of CD3 and CD28.

   Additional vials will be stored for RNA-sequencing of relevant populations identified in initial immunophenotyping.

   First point of contact
   Laura Sena
   CORUS Laura Sena
   Laura.sena@jhmi.edu
   410-440-5950

   Second point of contact
   Marc Rosen
   Rosenma@jhmi.edu

Table 1. Immuno- and metabolic phenotyping of PBMCs

Marker	Purpose
Live Dead	Dead cell exclusion
H3K27me3	Histone Methylation
CTLA4	Immune phenotype-Co-inhibitory

PD1	Immune phenotype-Co-inhibitory
41BB	Immune phenotype-Co-stimulatory
CCR7	Immune phenotype-Memory
CD45RA	Immune phenotype-Memory
Ki67	Immune phenotype-Proliferation
CD19	Immune subset-B cell
CD15	Immune subset-MDSC
CD33	Immune subset-MDSC
HLA-DR	Immune subset-Monocyte/DCs
CD14	Immune subset-Monocyte/MDSC
CD56	Immune subset-NK cell
CD3	Immune subset-T cell
CD4	Immune subset-T cell
CD8	Immune subset-T cell
FOXP3	Immune subset-Treg
CPT1a	Metabolic phenotype-Fatty acid oxidation
GLUT1	Metabolic phenotype-Glycolysis
Hexokinase II	Metabolic phenotype-Glycolysis
LDHA	Metabolic phenotype-Glycolysis
pS6	Metabolic phenotype-mTOR activity
TOMM20	Metabolic phenotype-OXPHOS
VDAC1	Metabolic phenotype-OXPHOS
AR	Androgen receptor

2. **Circulating cytokine and metabolite quantification** Hopkins/Sibley patients only

   Peripheral blood will be collected into **one purple-top EDTA tube** according to the study calendar at day 1 each OT cycle, C1 D8 of OT cycle, OT reassessment visit and Enzalutamide reassessment visit. Following collection, Laura Sena from the Denmeade lab will be notified for specimen transfer (see contact information as per PBMC studies). Sibley samples will be shipped to the Denmeade lab on same day as blood collection according to the instructions in the lab manual.

   Plasma will be isolated, aliquoted, and frozen at -80 degrees Celsius. Samples will be batch analyzed for measurement of cytokines by ELISA assay or metabolite levels by LC-MS.

3. **Body composition**

   Body composition will be determined by analysis of baseline and day 85 (end of OT

cycle three) CT scan using previously described methods (40). Subcutaneous fat, visceral fat and skeletal muscle area will be determined by analysis of CT scan at L3 level. Image analysis will be performed in collaboration with Dr. Gawandee, Department of Radiology, JHU.

13. References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics, 2008. CA Cancer J Clin. 2008;58:71-96.
2. Huggins C, Hodges CV (1941) Studies on prostatic cancer. I. The effect of castration, of estrogen and androgen injection on serum phosphatases in metastatic carcinoma of the prostate. Cancer Res 1(4): 293-297
3. Crawford ED, Eisenberger MA, McLeod DG, Spaulding JT, Benson R, Dorr FA, Blumenstein BA, Davis MA, Goodman PJ.A controlled trial of leuprolide with and without flutamide in prostatic carcinoma. N Engl J Med. 1989;321:419-24.
4. Laufer M, Denmeade SR, Sinibaldi VJ, Carducci MA, Eisenberger MA.Complete androgen blockade for prostate cancer: what went wrong? J Urol. 2000;164:3-9.
5. Linja MJ, Savinainen KJ, Saramaki OR, Tammela TL, Vessella RL & Visakorpi T 2001 Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. Cancer Res 61 3550-3555.
6. Shah RB, Mehra R, Chinnaiyan AM, Shen R, Ghosh D, Zhou M, Macvicar GR, Varambally S, Harwood J, Bismar TA, et al. Androgen-independent prostate cancer is a heterogeneous group of diseases: lessons from a rapid autopsy program. Cancer Res 2004;64:9209-9216.
7. de Bono JS, Logothetis CJ, Molina A, Fizaki K, North S, Chu L, Chi KN, Jones RJ, Goodman OB, Saad F, Staffurth JN, Mainwaring P, Harland S, Flaig TW, Hutson TE, Cheng T, Patterson H, Hainsworth JD, Ryan CJ, Sternberg CN, Ellard SL, Flechon A, Saleh M, Scholz M, Efstathiou E, Zivi A, Bianchini D, Loriot Y, Chieffo N, Kheoh T, Haqq CM, Scher HI, Cou-AA-301 Investigators. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med 2011;364:1995-2005. PMCID:PMC3471149
8. Scher HI, Fizazi K, Saad F, Taplin ME, Sternberg CN, Miller K, de Wit R, Mulders P, Chi KN, Shore ND, Armstrong AJ, Flaig TW, Fléchon A, Mainwaring P, Fleming M, Hainsworth JD, Hirmand M, Selby B, Seely L, de Bono JS; AFFIRM Investigators. Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med. 2012;367:1187-97.

9. Loriot Y, et al. Antitumour activity of abiraterone acetate against metastatic castration-resistant prostate cancer progressing after docetaxel and enzalutamide (MDV3100). Ann Oncol 2013;24:1807-12.
10. Noonan KL, North S, Bitting RL, Armstrong AJ, Ellard SL, Chi KN. Clinical activity of abiraterone acetate in patients with metastatic castration-resistant prostate cancer progressing after enzalutamide. Ann Oncol 2013;24:1802-7.
11. Schrader AJ, Boegemann M, Ohlmann CH, Schnoeller TJ, Krabbe LM, Hajili T, Jentzmik F, Stoeckle M, Schrader M, Herrmann E, Cronauer MV. Enzalutamide in Castration-resistant Prostate Cancer Patients Progressing After Docetaxel and Abiraterone. Eur Urol. 2013 Jul 2. [Epub ahead of print]
12. Bianchini D, Lorente D, Rodriguez-Vida A, Omlin A, Pezaro C, Ferraldeschi R, Zivi A, Attard G, Chowdhury S, de Bono JS. Antitumour activity of enzalutamide (MDV3100) in patients with metastatic castration-resistant prostate cancer (CRPC) pre-treated with docetaxel and abiraterone. Eur J Cancer. 2013 Sep 25. [Epub ahead of print]
13. Chen CD, Welsbie DS, Tran C, Baek SH, Vessella R, Rosenfeld MG, Sawyers CL. Molecular determinants of resistance to antiandrogen therapy. Nat Med 2004;10:33-9.
14. Isaacs JT, D'Antonio JM, Chen S, Antony L, Dalrymple SP, Ndikuyeze GH, Luo J, Denmeade SR. Adaptive auto-regulation of androgen receptor provides a paradigm shifting rationale for bipolar androgen therapy (BAT) for castrate resistant human prostate cancer. Prostate. 2012;72:1491-505. PMCID:PMC3374010
15. Dehm SM, Schmidt LJ, Heemers HV, Vessella RL, Tindall DJ. Splicing of a novel androgen receptor exon generates a constitutively active androgen receptor that mediates prostate cancer therapy resistance. Cancer Res. 2008;68:5469-77. PMCID:PMC2663383
16. Li Y, Chan SC, Brand LJ, Hwang TH, Silverstein KA, Dehm SM. Androgen receptor splice variants mediate enzalutamide resistance in castration-resistant prostate cancer cell lines. Cancer Res. 2013;73:483-9. PMCID:PMC3549016
17. Hu R, Dunn TA, Wei S, Isharwal S, Veltri RW, Humphreys E, Han M, Partin AW, Vessella RL, Isaacs WB, Bova GS, Luo J. Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. Cancer Res. 2009;69:16-22. PMCID: PMC2614301.
18. Sun S, Sprenger CC, Vessella RL, Haugk K, Soriano K, Mostaghel EA, Page ST, Coleman IM, Nguyen HM, Sun H, Nelson PS, Plymate SR. Castration resistance in human prostate cancer is conferred by a frequently occurring androgen receptor splice variant. J Clin Invest. 2010;120:2715-30. PMCID:PMC2912187
19. Linja MJ, Savinainen KJ, Saramaki OR, Tammela TL, Vessella RL, Visakorpi T. Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. Cancer Res 2001;61:3550-55.

20. Vander Griend DJ & Isaacs JT 2008 Androgen Receptor as a Licensing Factor for DNA Replication. In Androgen Action in Prostate Cancer. Eds DJ Tindall & JL Mohler: Springer, In Press.
21. Langeler EG, van Uffelen CJ, Blankenstein MA, van Steenbrugge GJ & Mulder E. Effect of culture conditions on androgen sensitivity of the human prostatic cancer cell line LNCaP. Prostate 1993;23:213-223.
22. Kokontis JM, Hay N, Liao S. Progression of LNCaP prostate tumor cells during androgen deprivation: hormone-independent growth, repression of proliferation by androgen, and role for p27Kip1 in androgen-induced cell cycle arrest. Mol Endocrinol 1998;12:941-953.
23. Kokontis J, Takakura K, Hay N, Liao S. Increased androgen receptor activity and altered c-myc expression in prostate cancer cells after long-term androgen deprivation Cancer Res. 1994;54:1566-73.
24. Umekita Y, Hiipakka RA, Kokontis JM, Liao S. Human prostate tumor growth in athymic mice: inhibition by androgens and stimulation by finasteride. Proc Natl Acad Sci U S A. 1996;93:11802-7.
25. Chuu CP, Hiipakka RA, Fukuchi J, Kokontis JM, Liao S. Androgen causes growth suppression and reversion of androgen-independent prostate cancer xenografts to an androgen-stimulated phenotype in athymic mice. Cancer Res. 2005 Mar 15;65:2082-4.
26. Litvinov IV, Vander Griend DJ, Antony L, Dalrymple S, De Marzo AM, Drake CG, Isaacs JT. Androgen receptor as a licensing factor for DNA replication in androgen-sensitive prostate cancer cells. Proc Natl Acad Sci U S A 2006;103: 15085-15090.
27. Vander Griend DJ, Litvinov IV, Isaacs JT. Stabilizing androgen receptor in mitosis inhibits prostate cancer proliferation. Cell Cycle 2007;6: 647-651
28. Haffner, MC, Aryree MJ, Toubaji A, Esopi DM, Albadine R, Gurel B, Isaacs WB, Bova GS, Liu W, Xu J, Meeker AK, Netto G, De Marzo AM, Nelson WG, Yegnasubramanian S. Androgen -induced TOP2B-mediated double-strand breaks and prostate cancer gene rearrangements. Nat Genet 2010;42:668-75. PMCID:PMC3157086
29. Hu R, Lu C, Mostaghel EA, Yegnasubramanian S, Gurel M, Tannahill C, Edwards J, Isaacs W, Nelson PS, Bluemn E, Plymate SR, Luo J. Distinct transcriptional programs mediated by the ligand-dependent full-length androgen receptor and its splice variants in castration-resistant prostate cancer. Cancer Res. 2012;72:3457-62. PMCID:PMC3415705
30. Thelen P, Heinrich E, Bremmer F, Trojan L, Strauss A. Testosterone boosts for treatment of castration resistant prostate cancer: An experimental implementation of intermittent androgen deprivation. Prostate 2013;73:1699-709.
31. Denmeade SR, Isaacs JT. Bipolar androgen therapy: the rationale for rapid cycling of supraphysiologic androgen/ablation in men with castration resistant prostate cancer. Prostate. 2010;70:1600-7.

32. Cai C, Chen S, Ng P, Bubley GJ, Nelson PS, Mostaghel EA, Marck B, Matsumoto AM, Simon NI, Wang H, Chen S, Balk SP. Intratumoral de novo steroid synthesis activates androgen receptor in castration-resistant prostate cancer and is upregulated by treatment with CYP17A1 inhibitors. Cancer Res. 2011 Oct 15;71(20):6503-13.
33. Brendler H, Chase WE, Scott WW. Prostatic Cancer: Further investigation of hormone relationships. Arch Surg 1949;433-440.
34. Prout GR, Brewer WR. Response of men with advanced prostatic carcinoma to exogenous administration of testosterone. Cancer 1967;20:1871-1878.
35. Szmulewitz R, Mohile S, Posadas E, Kunnavakkam R, Karrison T, Manchen E, Stadler WM. A Randomized Phase 1 Study of Testosterone Replacement for Patients with Low-Risk Castration-Resistant Prostate Cancer. Eur Urol. 2009 Feb 27. [Epub ahead of print]
36. Morris MJ, Huang D, Kelly WK, Slovin SF, Stephenson RD, Eicher C, Delacruz A, Curley T, Schwartz LH, Scher HI. Phase 1 trial of high-dose exogenous testosterone in patients with castration-resistant metastatic prostate cancer. Eur Urol. 2009 Aug;56(2):237-44. Epub 2009 Apr 3.
37. Hussain MH, Pienta KJ, Redman BG, Cummings GD, Flaherty LE. Oral etoposide in the treatment of hormone-refractory prostate cancer. Cancer. 1994;74:100-3.
38. Behre HM, Nieschlag E.Testosterone preparations for clinical use in males. In: Testosterone: Action, Deficiency, Substitution 4th Edition (E Nieschlag, H.M. Behre, eds.) Cambridge University Press, Cambridge UK, 2012, pp.309-35.
39. Simon R. Optimal two-stage designs for phase II clinical trials. Contr Clin Trials 1989; 10:1-10.
40. Marshall CH, Tunacao J, Danda V, Tsai HL, Barber J, Gawande R, Weiss CR, Denmeade SR, Joshu C. Reversing the effects of androgen-deprivation therapy in men with metastatic castration-resistant prostate cancer. BJU Int. 2021 Mar 25. doi: 10.1111/bju.15408

Appendix I

Response Evaluation Criteria in Solid Tumors (RECIST) Quick Reference

Eligibility

Only patients with measurable disease at baseline should be included in protocols where objective tumor response is the primary endpoint.

Measurable disease - the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology**.**

Measurable lesions - lesions that can be accurately measured in at least one dimension with longest diameter ≥20 mm using conventional techniques or ≥10 mm with spiral CT scan.

Non-measurable lesions - all other lesions, including small lesions (longest diameter <20 mm with conventional techniques or <10 mm with spiral CT scan), i.e., bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, and also abdominal masses that are not confirmed and followed by imaging techniques.

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

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

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

Methods of Measurement

CT and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT and MRI should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

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

When the primary endpoint of the study is objective response evaluation, ultrasound (US) should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

The utilization of endoscopy and laparoscopy for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in specialized centers. However, such techniques can be useful in confirming complete pathological response when biopsies are obtained.

Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response when all lesions have disappeared.

Cytology and histology can be used to differentiate between PR and CR in rare cases (e.g., after treatment to differentiate between residual benign lesions and residual malignant lesions in tumor types such as germ cell tumors).

Baseline Documentation of "Target" and "Non-Target" Lesions

All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total, representative of all involved organs, should be identified as ***target lesions*** and recorded and measured at baseline.

Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically).

A sum of the longest diameter (LD) for *all target lesions* will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor.

All other lesions (or sites of disease) should be identified as ***non-target lesions*** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

Response Criteria

Evaluation of Target Lesions

Complete Response (CR):	Disappearance of all target lesions
Partial Response (PR):	At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD
Progressive Disease (PD):	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions
Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

Evaluation of Non-Target Lesions

Complete Response (CR):	Disappearance of all non-target lesions and normalization of tumor marker level
Incomplete Response / Stable Disease (SD):	Persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits
Progressive Disease (PD):	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions

Although a clear progression of “non-target” lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed later on by the review panel (or study chair).

Evaluation of Best Overall Response

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having “symptomatic deterioration”. Every effort should be made to document the objective progression even after discontinuation of treatment.

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

Confirmation

The main goal of confirmation of objective response is to avoid over-estimating the response rate observed. In cases where confirmation of response is not feasible, it should be made clear when reporting the outcome of such studies that the responses are not confirmed.

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met. Longer intervals as determined by the study protocol may also be appropriate.

In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval (in general, not less than 6-8 weeks) that is defined in the study protocol

Duration of Overall Response

The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever status is recorded first) until the first date that recurrence or PD is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started.

Duration of Stable Disease

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

The clinical relevance of the duration of SD varies for different tumor types and grades. Therefore, it is highly recommended that the protocol specify the minimal time interval required between two measurements for determination of SD. This time interval should take into account the expected clinical benefit that such a status may bring to the population under study.

Response Review

For trials where response rate is the primary endpoint it is strongly recommended that all responses be reviewed by an expert(s) independent of the study at the study's completion. Simultaneous review of patients' files and radiological images is the best approach.

Reporting of Results

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data).

All of the patients who met the eligibility criteria should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered as failing to respond to treatment (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

All conclusions should be based on all eligible patients.

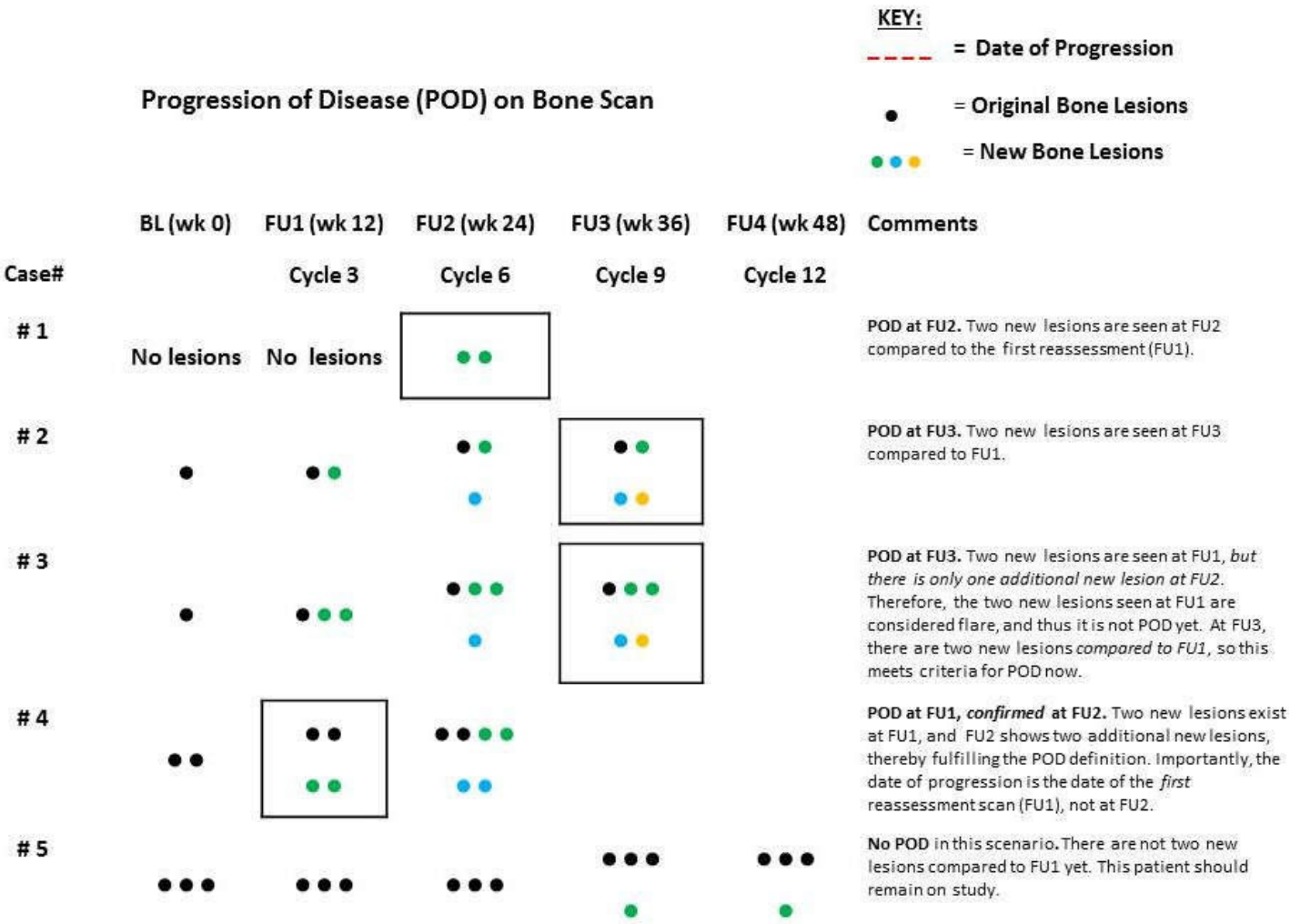
Sub-analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these sub-analyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported.

The 95% confidence intervals should be provided.

Appendix II
NCI COMMON TOXICITY CRITERIA, VERSION 4.03

Version 4.03 of the NCI CTCAE, dated 14 June 2010, may be viewed and/or downloaded by accessing the following website:
https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

Appendix 3: Bone Scan Assessment



Appendix 4: FACIT Fatigue Scale (Version 4)

Appendix 5: SF-36 Health Survey

Appendix 6: Serious Adverse Event Reporting Form