A Randomized Phase II Study Comparing Bipolar Androgen Therapy vs. Enzalutamide in Asymptomatic Men with Castration Resistant Metastatic Prostate Cancer:

The TRANSFORMER Trial
(Testosterone Revival Abolishes Negative Symptoms, Fosters Objective Response and Modulates Enzalutamide Resistance)

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SYNOPSIS:

Title: A Randomized Phase II Study Comparing Bipolar Androgen Therapy vs. Enzalutamide in Asymptomatic Men with Castration Resistant Metastatic Prostate Cancer: The TRANSFORMER Trial

Objective: The primary objective of the study is to determine if treatment with supraphysiologic testosterone (T) will improve progression free survival compared to enzalutamide in men with metastatic castrate-resistant prostate cancer (CRPC) post-treatment with abiraterone.

Study Design: Asymptomatic men with progressive metastatic CRPC post- treatment with abiraterone acetate (pre-chemotherapy for metastatic disease) will be treated on a randomized, multi-Institutional open label study to determine if treatment with intramuscular T given on a dose/schedule designed to result in rapid cycling from the polar extremes of supraphysiologic to near castrate levels [i.e. Bipolar Androgen Therapy (BAT)] will improve primary and secondary objectives vs. enzalutamide as standard therapy.

Treatment Plan: Eligible patients will have metastatic CRPC with no disease related symptoms and progression on ADT and will have progressed post-treatment with abiraterone. Patients will continue on ADT with LHRH agonist (i.e. Zoladex, Trelstar, Eligard or Lupron) or LHRH antagonist (Degarelix) if not surgically castrated throughout the duration of the study to inhibit endogenous testosterone production. Patients will be randomized 1:1 and stratified based on duration of prior abiraterone acetate therapy (6 months or less or greater than 6 months). Patients randomized to BAT (Arm A) will receive intramuscular injections with either testosterone cypionate or testosterone enanthate at a dose of 400 mg every 28 days. This dose was selected based on data demonstrating that it produces an initial supraphysiologic serum level of T (i.e. > 1500 ng/dL or 3-10 times normal level) with eugonadal levels achieved at the end of two weeks and near castrate levels after 28 days. Patients randomized to enzalutamide (Arm B) will receive daily oral dose of 160 mg. Each cycle is defined as 28 days.

Patients will have PSA level and symptoms assessment checked every cycle. Every 3 cycles patients will have repeat bone/CT scans to evaluate treatment response status. On CT scan, radiographic progression will be defined by RECIST criteria (i.e. >20% increase in the sum of target lesions). On bone scan, radiographic progression will be defined by PCWG2 criteria as ≥ 2 new bone lesions (Appendix 7). However, for the first reassessment scan only, patients should remain on study 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 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 (Appendix 7).

Patients with PSA progression but with disease response or stable disease on imaging studies will remain on study until radiographic or other clinical progression criteria are met. Patients with radiographic disease progression will not receive continued BAT (arm A) or enzalutamide (arm B) and will be eligible for crossover to the opposite therapy. Patients on the BAT arm A can cross over to receive enzalutamide at time of progression or can choose to go off study and be treated with other standard of care treatments. Patients on the enzalutamide arm B will be allowed to cross-over to receive BAT or can choose to go off study and be treated with other standard of care treatments. Patients with clinical progression due to prostate cancer must meet study exclusion criteria to be permitted to cross-over to the opposite treatment. Patients with clinical progression due to pain from prostate cancer are not permitted to cross-over.

Study Population: Men with CRPC with progressive disease (radiographically and/or biochemically)

who have been treated with continuous castrating androgen ablative therapy and abiraterone.

Number of Patients: 194 (97 per treatment arm)
Inclusion criteria:

1. ECOG Performance status ≤2.
2. Age ≥18 years.
3. Histologically-confirmed adenocarcinoma of the prostate.
4. Treated with continuous androgen ablative therapy (either surgical castration or LHRH agonist/antagonist).
5. Documented castrate level of serum testosterone (<50 ng/dl).
6. Metastatic disease radiographically documented by CT/MRI or bone scan.
7. Must have had disease progression while on abiraterone acetate alone or abiraterone acetate in combination with other investigational agents based on:
    - PSA progression defined as an increase in PSA, as determined by 2 separate measurements taken at least 1 week apart

    And/ Or
    - Radiographic disease progression, based on RECIST 1.1 in patients with measurable soft tissue lesions or PCWG2 for patients with bone disease
8. Screening PSA must be ≥ 1.0 ng/mL.
9. Prior treatment with additional second line hormone therapies is allowed.
10. No prior treatment with enzalutamide, ARN-509, ODM-201, galeterone or other investigational AR targeted treatment is allowed.
11. Prior docetaxel for hormone-sensitive prostate cancer is permitted if ≤ 6 doses were given in conjunction with first-line androgen deprivation therapy and >12 months since last dose of docetaxel.
12. Prior treatment with Provenge vaccine and ^{223}Radium (Xofigo) is allowed if >4 weeks from last dose.
13. Patients must be withdrawn from abiraterone for ≥ 2 weeks.
14. Patients must be weaned off prednisone and be off therapy for ≥ 1 week prior to starting therapy.
15. Acceptable liver function:
    a. Bilirubin < 2.5 times institutional upper limit of normal (ULN)
    b. AST (SGOT) and ALT (SGPT) < 2.5 times ULN
16. Acceptable renal function:
    a. Serum creatinine < 2.5 times ULN
17. 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.
18. At least 4 weeks since prior radiation.
19. Ability to understand and willingness to sign a written informed consent document.
20. Patients on either treatment arm will be considered for crossover if they demonstrate evidence of radiographic disease progression from the initial treatment.

Exclusion criteria:

1. Pain due to metastatic prostate cancer requiring treatment intervention.
2. ECOG Performance status ≥3
3. Prior treatment with enzalutamide is prohibited.
4. Prior treatment with docetaxel or cabazitaxel for metastatic castration-resistant prostate cancer is prohibited.
5. Requires urinary catheterization for voiding due to obstruction secondary to prostatic enlargement well documented to be due to prostate cancer or benign prostatic hyperplasia (BPH).
6. 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, severe and extensive spinal metastases with concern over spinal cord compression, extensive liver metastases).
7. 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.
8. Active uncontrolled infection, including known history of HIV/AIDS or hepatitis B or C.
9. Any psychological, familial, sociological, or geographical condition that could potentially interfere with compliance with the study protocol and follow-up schedule.
10. Patients receiving anticoagulation therapy with Coumadin are not eligible for study. [Patients on non-coumadin anticoagulants (Lovenox, Xarelto, etc.) are eligible for study. Patients on Coumadin who can be transitioned to lovenox prior to starting study treatments will be eligible].
11. Patients with prior history of a thromboembolic event within the last 12 months that is not being treated with systemic anticoagulation are excluded.
12. Patients allergic to sesame seed oil or cottonseed oil are excluded.
13. Major surgery (eg, requiring general anesthesia) within 3 weeks before screening, or has not fully recovered from prior surgery (ie, unhealed wound). Note: subjects with planned surgical procedures to be conducted under local anesthesia may participate.

Study Endpoints:

PRIMARY:	PROGRESSION FREE SURVIVAL
SECONDARY:	PSA RESPONSE
	SAFETY
	TIME TO PSA PROGRESSION
	RADIOGRAPHIC PROGRESSION FREE SURVIVAL
	TIME TO DOCETAXEL CHEMOTHERAPY
	MEASURABLE DISEASE RESPONSE
	PSA RESPONSE TO ENZALUTAMIDE POST-BAT THERAPY
	PSA RESPONSE TO BAT POST-ENZALUTAMIDE THERAPY
	OVERALL SURVIVAL

PFS2 (Time from initiation of therapy to progression on crossover treatment

QUALITY OF LIFE

EFFECT OF BAT VS. ENZALUTAMIDE ON EXPRESSION OF AR-V7 AND FREQUENCY OF AR-MUTATION.

Statistical Plan:

Primary objective and primary endpoint: The primary objective of the study is to compare progression-free survival (PFS) between BAT and enzalutamide in patients with castration resistant prostate cancer who have progressed on abiraterone. The primary efficacy endpoint is PFS, defined as the time from the date of the randomization to the date of first documented clinical progression due to prostate cancer or radiological confirmation of progression per RECIST 1.1 for soft tissue or PCWG2 for bone lesions or death, whichever occurs first. If a patient has not had the event at the date of the analysis cut-off, PFS will be censored at the time of the last adequate tumor assessment before the cut-off date.

Analysis of primary endpoint: The comparison of PFS will be an intent-to-treat analysis including all randomized patients. The PFS will be estimated using Kaplan-Meier method, and the median PFS along with 95% confidence intervals (CI) will be reported by the treatment groups. The PFS will be compared between the two arms using a stratified log-rank test with the stratification factor of duration of prior abiraterone treatment. The Cox regression model, stratified for the same baseline stratification factors, will be used to estimate the hazard ratio (HR) of PFS between the two arms and corresponding 95% CI. Additionally, a Cox regression model, stratified for the baseline stratification factors, will be used to explore the potential influences of the other factors on the primary endpoint PFS.

Sample size and power considerations: Based on published results, radiographic progression in the enzalutamide arm post abiraterone will be detected in most patients during the first 6 months of treatment and hence we selected the 6 months PFS target as a reasonable endpoint 6 months. This trial is designed to detect a 50% improvement in median PFS in the BAT group, from 6 to 9 months (corresponding to a HR 0.667 of BAT vs. enzalutamide), at one-sided significance level of 0.05. The study requires at least 156 events to ensure a sequential test procedure will have 80% power. We expect a recruitment period of 24 months, 24 months and an additional 12 months of follow-up. After accounting for an estimated 15% loss to follow-up, a total of up to 194 patients (97 per arm) will be randomized to record 156 events in 36 months of study. Recruitment for the study will stop once a total of 156 events have occurred.

Study Schema:

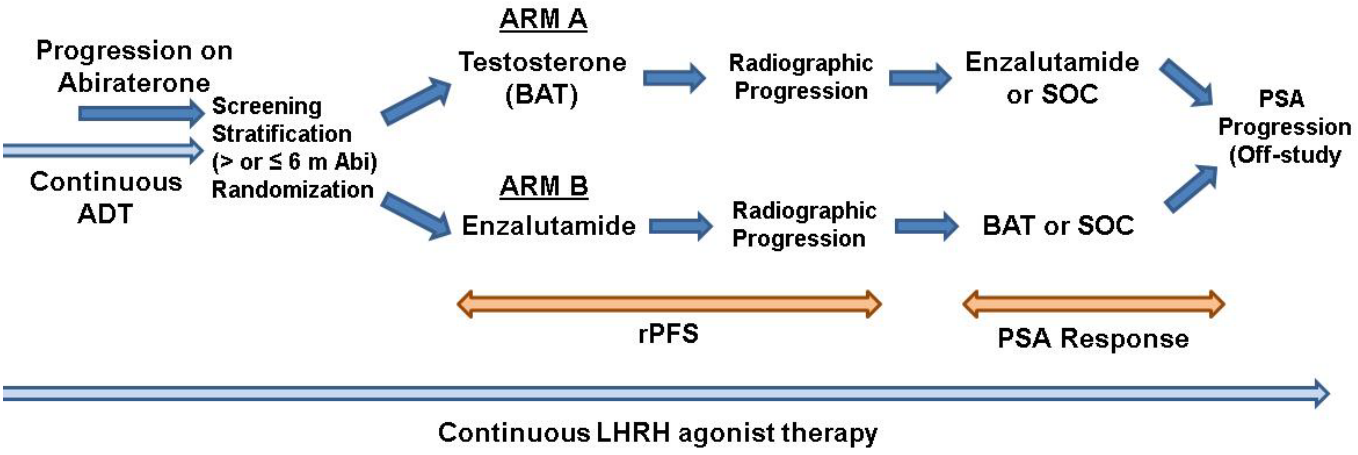


TABLE OF CONTENTS

1. INTRODUCTION

1.1. Overview and Rationale

Prostate cancer is uniformly lethal once it has escaped the confines of the prostate gland, resulting in the death of over ~30,000 American men each year (1). Androgen ablation therapy has remained the standard of care for men with recurrent/metastatic cancer since its discovery by Charles Huggins in the 1940s (2). While androgen ablation therapy provides significant palliative benefit, all men undergoing androgen ablation eventually relapse and no longer respond to androgen ablation no matter how completely given(3,4).

This observation led to the labeling of patients progressing on androgen ablative therapies as having "androgen independent" or "hormone refractory" prostate cancer. However, new findings have demonstrated that the majority of prostate cancer specimens from androgen ablated patients continue to express the androgen receptor (AR) often at higher levels (5, 6). In addition, variants of AR that do not bind to ligand also are upregulated in androgen deprived prostate cancer cells. Prostate cancer cells from these castration refractory patients continue to express AR-regulated genes such as PSA. This observation has resulted in a reclassification of "hormone refractory" disease as "Castration Resistant Prostate Cancer" (CRPC) and has opened up new avenues of research into the function of the AR in the androgen deprived state. These findings suggest that "castration-resistant" prostate cancer may continue to survive through aberrant AR signaling. Studies have demonstrated adaptation to chronic androgen deprivation through several mechanisms including marked upregulation of the full length AR, AR gene amplification, and expression of AR splice variants lacking the ligand binding domain (7-10). This observation has led to a renewed interest in the AR axis as a therapeutic target. On this basis, enzalutamide, a new antiandrogen, and abiraterone acetate, a CYP17 androgen synthesis inhibitor, have recently been approved as second line therapy for prostate cancer on the basis of modest observed improvements in overall survival vs. placebo in randomized phase III trials (11-14).

A major mechanism for the development of CRPC following chronic exposure to androgen ablative therapies is the ability of prostate cancer cells to adapt to the lack of ligand by marked upregulation of the full length AR and AR splice variants lacking the ligand binding domain. AR gene amplification is also commonly seen in samples from patients on chronic androgen deprivation. Laboratory studies have documented this upregulation of AR. These studies have demonstrated that this upregulation of AR may be responsible for the resistance to antiandrogens. In these studies, these re-exposure of androgen starved prostate cancer cells readapt upon exposure to androgen by lowering AR expression. This lowered AR expression now re-sensitizes these cells to androgen ablative therapies such as antiandrogens (9, 10).

In this background of renewed interest in blocking the AR, there has been the paradoxical observation that the growth of both androgen sensitive and androgen resistant prostate cancer cell lines is inhibited by the addition of testosterone or other synthetic androgens to the media (10,15-17). Typically, in vitro data in human prostate cancer cell lines demonstrates a biphasic response to androgens, with very low levels producing modest growth stimulation and expression of prostate tissue differentiation markers such as PSA, while higher levels of androgen in the media suppress growth and PSA production (10, 15-17). High levels in this case can be as low as picomolar concentrations suggesting that these "androgen ablation resistant" cells are exquisitely sensitive to androgens. These in vitro studies are supported by animal studies that have demonstrated that androgen receptor positive human prostate cancer cells selected to grow in castrated animals' upregulate androgen receptor levels. Similar to the in vitro response, in these

models, systemic testosterone administration produces significant growth inhibition, whereas antiandrogens such as bicalutamide promote prostate cancer growth.

Until recently, the mechanisms underlying this paradoxical response have been unknown. However, recent data from our group and others have described several possible mechanisms for this effect of androgens on the growth of CRPC cells. The androgen receptor has been shown to be a licensing factor involved in DNA relicensing during progression through the cell cycle (14,16). AR is degraded as the prostate cancer cell goes through cycle. We have demonstrated that the high levels of AR seen in CRPC cells do not get sufficiently degraded in the presence of supraphysiologic androgen due to androgen stabilization of the AR. Thus, under these conditions, AR remains bound to origins of replication preventing the cell from progressing through subsequent cell cycles and ultimately resulting in cell death. In addition, it has been demonstrated that replenishment of androgen to androgen starved prostate cancer cells rapidly produces significant double strand DNA breakage that can result in inhibition of growth, inhibition of protein synthesis, growth and loss of clonogenic survival (18). Finally, androgen starved cells upregulate constitutively active AR splice variants that cannot bind androgen due to loss of the ligand binding domain (19,20). CRPC cells may rely on these truncated AR variants for survival under low ligand conditions. However, it has been shown that when androgen starved CRPC cells are given high dose androgen, expression of these variants is rapidly downregulated to often undetectable levels (21, 22).

On the basis of these observations, the hypothesis of this trial is that a significant clinical response can be achieved in men with long standing castration resistant prostate cancer by rapidly cycling from the polar extremes of supraphysiologic to castrate serum levels of androgen. We have termed this approach Bipolar Androgen Therapy (BAT) (14). By pursuing this treatment strategy, high AR–expressing CRPC cells will be sensitive to killing by supraphysiologic levels of testosterone according to mechanisms described above. Those cells that try to adapt to high androgen by dropping AR expression to low levels will then become sensitive to killing when testosterone levels are lowered to near castrate levels.

1.2. Androgen Ablative Therapy for Prostate Cancer

The current treatment approach for CRPC: Men with recurrent or metastatic prostate cancer are initially treated with LHRH agonists which typically result in castrate levels of serum T (i.e. <50 ng/dL) within 2-4 weeks post initiation of therapy (24). The catastrophic loss of androgen as their major growth and survival factor results in the death of the majority of prostate cancer cells. On this basis, the majority (~90%) of men have an initial beneficial palliative response to ADT. However, relapse occurs in all men treated with ADT. Over time prostate cancer cells that survive the initial acute drop in serum androgen adapt to the chronic low androgen conditions by upregulating AR through overexpression, gene amplification and expression of truncated, transcriptionally active AR splice variants (AR-V) that lack the ligand binding domain. The first clinical manifestation of this adaptive increase in AR signaling is the renewed production of PSA. At this point the patient is considered to have CRPC. Typically, this patient would continue on ADT and begin second-line hormonal therapies. This approach is based on the concept that a sufficient amount of androgen is produced by the adrenal glands and perhaps by the prostate cancer cells themselves (18) to support the growth of the surviving adapted CRPC cells. Thus, second line hormonal therapies were developed that either competitively inhibit androgen binding to AR (e.g. anti-androgens flutamide, bicalutamide, nilutamide) or inhibit adrenal androgen synthesis (e.g. ketoconazole) (24). While clinical benefit was demonstrated, until recently, the effect of second-line therapy on survival was unknown due to lack of appropriately powered studies. However, recently enzalutamide and abiraterone both received FDA-approval for use in metastatic CRPC based on a modest survival benefit observed in large randomized studies, Table 1 (11-14).

The current treatment approach for CRPC is to continue chronic LHRH agonist therapy despite progression and administer “second line” hormone therapy. Based on a demonstrated survival benefit, Abiraterone is emerging as the preferred initial second line therapy. Before the availability of Enzalutamide, standard therapy in men with progression on Abiraterone would be to give docetaxel chemotherapy. Currently, Enzalutamide is FDA-approved for use in men post docetaxel based on Phase III results showing an ~ 5 month improvement in survival in the post-docetaxel setting, Table 1 (8). However, Enzalutamide is frequently being administered to men prior to docetaxel if insurance clearance can be obtained. It is also expected that Enzalutamide, like Abiraterone, will be approved for use in the pre chemotherapy setting based on positive results from the PREVAIL study that showed an improvement in median overall survival that was estimated at 32.4 months in the enzalutamide group and 30.2 months in the placebo group (hazard ratio, 0.71; 95% CI, 0.60 to 0.84; P<0.001) (10).

Thus, based on the result of these studies, the ease of administration of these oral agents, and the possibility of delaying the need for chemotherapy, the evolving treatment paradigm will likely involve the sequential addition of Abiraterone and Enzalutamide to LHRH agonist-based ADT in men with ADT progression. However, several small studies have demonstrated that sequential use these agents in the post-chemotherapy setting is associated with significant reduction in time to radiographic progression to <5 months, decreased PSA response and objective response suggesting cross-resistance between these agents, table 1 (23-6). Limited information is available on progression free survival with enzalutamide post-abirateroner in the pre-chemotherapy setting but data on small number of patients indicates median rPFS ≤ 6 mos (27,28). The mechanisms underlying this reduced response rate are likely multi-factorial and may include continued adaptive increase in AR expression, increased expression of ligand independent AR variants in resistant cells and emergence of AR mutations that may affect enzalutamide binding.

Table 1. Results from sequential treatment with Abi or Enza in CRPC

	N=	Overall Survival (m)	>50% decline PSA (%)	TTP (m)	Objective Response (%)
Abiraterone Phase III (post-chemo)	1195	15.8	29.5	8.5	14
Abiraterone post Enzalutamide	38	7.2	8	2.7	8
Abiraterone post Enzalutamide	27	11.7	3	3.5	0
Enzalutamide Phase III (post-chemo)	1199	18.4	54	8.3	29
Enzalutamide post Abiraterone	35	7.1	28.6	4.9	2.9
Enzalutamide post Abiraterone	39	NR	12.8	2.8	4.3
Enzalutamide post Abiraterone	41	9.4	19.6	4.2	5.9

1.3. AR-splice variants in CRPC

The AR protein contains several functional domains (29). The N-terminal domain (NTD), encoded by exon 1, constitutes ~60% of the 110-kDa full-length protein and is the transcriptional regulatory region of the protein. The central DNA-binding domain (DBD) is encoded by exons 2 and 3, whereas exons 4 to 8 code for the highly conserved C-terminal ligand-binding domain (LBD) which is the intended target of all current existing AR-directed therapies (29). Recently, AR variant (AR-Vs) transcripts that are encoded by aberrantly spliced AR mRNA have been discovered that lack the reading frames for the ligand-binding domain due to splicing of “intronic” cryptic exons to the upstream exons encoding the AR DNA-binding domain (29). A total of 15 AR-Vs have been fully decoded, with the variant AR-V7 representing the single most important AR-V for which expression levels of its mRNA and protein can be detected in the vast majority of clinical CRPC specimens by variant-specific probes and antibodies (20, 29).

To evaluate mRNA expression of full length AR and AR-V7 in CTC we modified the AlereTM CTC AdnaTest, a commercial diagnostic test in CE-marked countries. The ProstateCancerSelect kit was used to enrich circulating prostate tumor cells in the blood using magnetic beads coated with 3 different antibodies (EPCAM and 2 proprietary antibodies). Cell capture by the optimized antibody combination is followed by cell lysis and RT-PCR analysis using a combination of multiple mRNA markers of the AR axis, using the ProstateCancerDetect kit and custom probes. We have developed specific probes to detect both the canonical AR-FL and AR-V7 that both increase upon treatment with Enzalutamide and Abiraterone in cell line and xenograft models (29). We used this test and methodology to interrogate CTCs for the presence or absence of AR-V7 from prospectively enrolled patients with metastatic castration-resistant prostate cancer initiating treatment with either enzalutamide or abiraterone (30). We examined associations between AR-V7 status and PSA response rates, PSA-progression-free survival (PSA-PFS), and clinical/radiographic progression-free survival (PFS). Multivariable Cox regression analyses were performed to determine the independent effect of AR-V7 status on clinical outcomes (30).

Thirty-one enzalutamide-treated patients and thirty-one abiraterone-treated patients were enrolled, of which 38.7% and 19.4% had detectable AR-V7 from CTCs, respectively (30). Among men receiving enzalutamide, AR-V7–positive patients had inferior PSA response rates (0% vs 52.6%, P=0.004), PSA-PFS (median: 1.4 vs 5.9 months, P<0.001), and PFS (median: 2.1 vs 6.1 months, P<0.001) compared to AR-V7–negative patients. Similarly, among men receiving abiraterone, AR-V7–positive patients had inferior PSA response rates (0% vs 68.0%, P=0.004), PSA-PFS (median: 1.3 months vs not reached, P<0.001), and PFS (median: 2.3 months vs not reached, P<0.001). The negative prognostic impact of AR-V7 detection was confirmed in multivariable analyses (30). The conclusion from this study is that the presence of AR-V7 in CTCs from patients with castration-resistant prostate cancer predicts resistance to enzalutamide and abiraterone (30).

Recently, Hu et al demonstrated that human CRPC cells LNCaP95 and VCaP rapidly downregulate expression of all AR isoforms following exposure to high dose androgen (21) suggesting a potential for re-sensitization to anti-androgens following exposure to supraphysiologic-T. Androgen exposure resulted in rapid decrease or loss of AR-V7 nuclear staining in these cell lines. These results were further confirmed by Thelen et al, who demonstrated that androgen treatment of the AR-V7 overexpressing human prostate cancer cell line VCaP results in rapid downregulation of total AR levels and almost complete loss of AR-V7 expression (22). These AR-V expressing cells are profoundly growth inhibited by supraphysiologic levels of androgens, Figure 1A. In contrast, these lines are highly resistant to the anti-androgen bicalutamide. We also observed complete loss of AR-V expression in VCaP cells over a 48 hr exposure to the synthetic androgen R1881, Figure 1B.

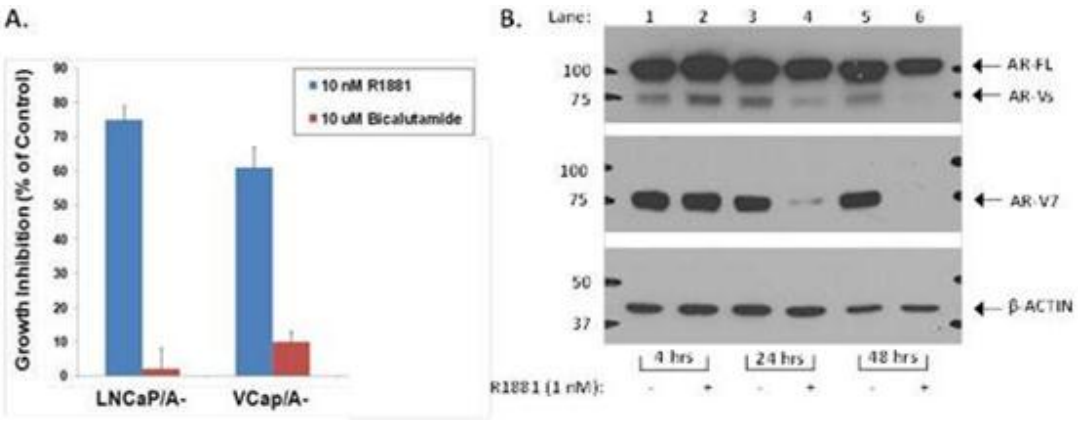


Figure 1. (A) Effect of synthetic androgen R1881 or bicalutamide on growth of ADT-resistant LNCaP and VCAP; (B) Expression of full length AR (AR-FL), AR-variants (AR-Vs) using antibody to AR N-terminal domain and AR-V& using AR-V7 specific antibody in the absence or presence of R1881

On the basis of these pre-clinical and clinical results, we will use the AlereTM CTC AdnaTest to isolate blood samples from patients on each arm at screening, after three months on BAT or Enzalutamide and at time of radiographic progression to determine the effect of each treatment arm on the expression of full length AR and AR-V7.

1.4. Preliminary Data in Support of Testosterone Therapy in CRPC

1.4.1. Androgen Produces Double Strand Breaks in Human Prostate Cancer Cells

The mechanisms underlying the growth suppressive effects of high levels of androgens in prostate cancer cells in vitro and in vivo is likely highly complex. Recent evidence from our group suggests that one mechanism may involve the formation of androgen induced Topoisomerase II beta (TOP2B) mediated double strand breaks at AR target genes, Figure 2(18). Recent studies have shown that estrogen signaling in breast cancer cells involves the co-recruitment of Estrogen receptor and TOP2B to estrogen receptor target sites, where TOP2B introduces transient double strand breaks. We hypothesized that such a mechanism may be involved in androgen signaling in prostate cancer cells and that at high doses of androgens, such breaks may persist and ultimately lead to growth suppression. In support of this, we observed that stimulation of androgen-deprived LNCaP cells with dihydrotestosterone (DHT) led to recruitment and catalytic activity of TOP2B at AR target sites in the TMPRSS2 enhancer as well as at other known AR target sites. At high doses of DHT, this TOP2B recruitment and catalytic activity was associated with significant formation of AR and TOP2-dependent persistent double strand breaks at the TMPRSS2 gene, as observed by fluorescence in situ hybridization (FISH) assay capable of detecting genomic breaks on an individual cell basis (18). Such breaks likely occurred throughout the genome at AR target sites since we observed numerous γH2A.x foci, a marker for double strand break formation, throughout the nucleus in response to stimulation of LNCaP cells with high-dose DHT (Figure 2). In further confirmation of this, we also observed recruitment of ATM, a double strand break repair signaling protein, to AR target sites in PSA and TMPRSS2, genes present on different chromosomes in the cell. These findings suggest that exposure of prostate cancer cells from patients with CRPC to high doses of testosterone may induce growth suppression due to the accumulation of androgen-mediated, TOP2-induced double strand DNA breaks (18).

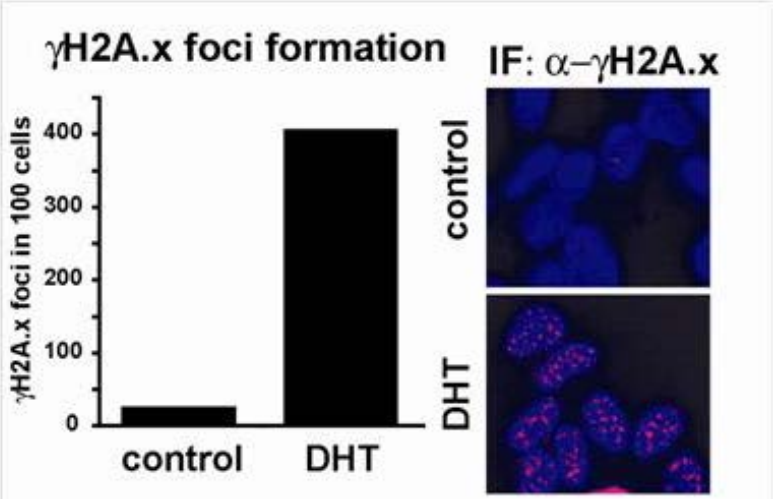


Figure 2. Androgen induced double strand DNA breaks in prostate cancer cell lines stimulated with high levels of androgen. Stimulation of androgen deprived LAPC4 cells (control) to high levels of DHT (DHT) leads to numerous double strand breaks throughout the nucleus as evidenced by the accumulation of numerous γH2A.x foci, a marker for formation of double strand breaks.

1.4.2. Adaptive Auto-regulation of AR Leads to Over-stabilization of the AR at Origins of Replication When Exposed to Supraphysiologic Androgen Levels

Recently, our laboratory has explored a mechanism for growth inhibition by T in CRPC cells (14,16,17). These studies document that the increase in AR expression observed in these cells in the low T environment creates a unique therapeutic vulnerability to selectively kill CRPCs. This is based upon the fact that AR is involved in DNA relicensing and DNA replication AR must degraded each cell cycle for proper relicensing to occur. Overstabilization of the increased levels of AR observed in CRPC with supraphysiologic testosterone prevents complete degradation of AR via the proteasome during mitosis (14,16,17). This was demonstrated by in vivo treatment of resistant human LNCaP prostate cancer xenografts with T implants to achieve supraphysiologic serum T-levels (14). This treatment resulted in significant growth inhibition, Figure 3a. These growth inhibited cells had similar amount of cells with nuclear AR in the nucleus, and Ki-67 positivity, Figure 3a. However, in xenografts treated with supraphysiologic T, the Cell Death Index was ~ 3-fold higher (14). More strikingly, the percent of cells staining positive for AR in mitosis was approximately 10-fold higher in cells exposed to supraphysiologic T vs. castrate only animals, Figure 3b. This data suggests that CRPC cells that have not properly relicensed DNA can die when they attempt to proceed through a subsequent cell cycle. Thus, based on this proposed mechanism, prostate cancer cells that maintain high AR levels will be vulnerable to cell death when exposed to supraphysiologic T conditions due to inability to rapidly auto-regulate AR to lower levels. Due to the bipolar cycling between high and low serum T achieved with BAT, those cells that do manage to survive the high T environment through adaptive down-regulation of AR will become vulnerable to cell death when suddenly exposed to low T conditions that occur over the cycle of BAT.

A.

	Castrate	Castrate + T implants
Tumor Volume (cc)	0.98 ± 0.17	0.32 ± 0.06*
PSA/gram tumor	24.5 ± 9.2	95.5 ± 5.4*
Ki-67	>75%	>75%
Mitotic Index	0.51 ± 0.15%	0.32 ± 0.23%
AR in Nucleus	>90%	>90%
Cell Death Index	15 ± 8%	39 ± 9%*
AR in Mitosis	7 ± 6%	77 ± 8%*

* $p<0.05$

B.

Figure 3: Castrated NOG mice inoculated with LNCaP/A-cells were either exposed to BAT therapy (via an implanted testosterone filled capsule that was placed and removed at two week intervals) or left in a permanently castrate state (diamond versus box). (A) Evaluation of indicated parameters in LNCaP/A- cells growing in castrate mice vs. castrate mice supplemented with subcutaneous testosterone-filled silastic implants. (B) Immunohistochemical staining for AR in harvested LNCaP/A- xenografts growing in castrate vs. castrate + T-pelleted mice. Blue arrows indicate mitotic figures.

1.5. Clinical Experience with Testosterone in Prostate Cancer:

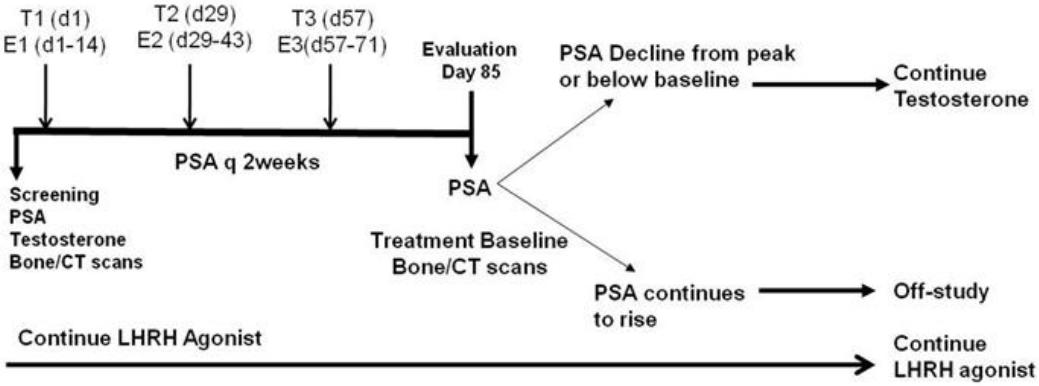
Up until recently, there had been very limited clinical experience in the PSA-era treating CRPC patients with testosterone. Brendler et al at the Brady Urological Institute reported in the Archives of Surgery in 1949 on the use of parenteral testosterone in several men with advanced CRPC (31). They observed considerable improvement in several men that included decreased pain, decreased prostate size and decreases in acid and alkaline phosphatase. In a second study, Prout and Brewer reported in Cancer in 1967 on the treatment of men who had been either untreated or recently castrated or long term castrates with parenteral testosterone (32). Five relapsed patients in the long term castrate group received testosterone for at least one month and 4 of 5 had subjective improvement. Five remaining patients in the long term castrate group received testosterone for 1-19 days, with each progressing and subsequently coming off therapy. Acid phosphatase declined in 2/5 men receiving a longer course of testosterone. Remarkably, one man in this group admitted to hospital with severe back pain, weakness and anorexia had a 10 month response with complete cessation of pain, excellent appetite and weight gain with decrease in acid phosphatase from 50 to 5 units.

In contrast, a number of studies during the 1960-70s evaluated the use of T-priming in combination with ^{32}P-sodium phosphate to treat men with CRPC and severe pain due to widespread bony metastases (33, 34). In these studies, initial T-priming using a variety of parenteral dosing regimens was associated with transient increase in bone pain during the first week followed by excellent pain palliation following administration of ^{32}P. Similar results were observed in studies led by Manni who evaluated T- priming with chemotherapy in the 1980's (35). These studies were also conducted in men with CRPC and pain due to widely metastatic disease. In these studies, increased bone pain was also observed in men upon initial treatment with oral androgens. The increased pain in these studies typically occurred within days of T administration. Thus, given this time frame, it is likely the increased pain was due to T-stimulation of inflammation/cytokine release within sites of bone metastases rather than a direct effect on tumor growth. Such rapid change is also seen in men with bone pain upon initially starting ADT. Marked improvement in pain after ADT often occurs within hours of treatment, an effect not due to tumor death but rather a rapid change in expression of pain-promoting gene products.

More recently, two Phase I studies were reported describing the results of the use of transdermal T as therapy for men with CRPC who had minimal to moderate disease burden and no pain due to prostate cancer. In the first study, Szmulewitz et al evaluated the effect of increasing doses of transdermal T in 15 men with early CRPC (rising PSA and minimal bone disease) (36). Five men each were treated with 2.5, 5.0 or 7.5 mg/day of transdermal T which brought the median concentration of T from castrate to 305, 308 and 297 ng/dl respectively. In this study no grade 3 or 4 toxicities were observed with the exception of one man who was taken off study at week 53 for grade 4 cardiac toxicity. Only one patient had symptomatic progression and three patients (20%) had a decrease in PSA (largest was 43%). Patients treated at the highest T dose had a prolonged time to progression that did not reach statistical significance most likely due to the small cohort size. In the second study, Morris et al evaluated the effect of transdermal T at a dose of 7.5 mg/day administered for 1 week (n=3), 1 month (n=3) or until disease progression (n=6) in 12 patients with CRPC (37). They observed no grade 3 or 4 toxicities and no pain flares. Eugonadal serum T levels were reported for this study. No objective responses were observed. Four patients had at least 20% declines and one achieved a >50% PSA decline.

Neither of these Phase I studies achieved the supraphysiologic levels of serum T that can be reached with FDA-approved doses of T administered as an intramuscular depot (38). However, the levels of serum T achieved were in the high-end of the eugonadal range. Remarkably, although the studies were considered “negative” from the standpoint of disease response, in both studies the administration of parenteral T to men with CRPC was very well-tolerated and did not result in significant worsening of disease or symptoms, including pain flares. While only one patient out of 27 from the combined studies had a reported >50% decline in PSA, smaller PSA declines were observed in a few of the patients on these two studies with a trend toward a dose-responsive effect, suggesting a potential for therapeutic benefit in some patients (36, 37).

A.



B.

Median age, yrs (range)	71 (56-87)
Median PSA, ng/mL (range)	20.0 (1.4-819.1)
Median testosterone, ng/dL (range)	<20 (<20-41)
Gleason score, N (%)	
6	4 (25)
7	7 (43.8)
8	3 (18.8)
9	2 (12.5)
Median length of continuous ADT, mos (range)	45.5 (12-146)
ECOG performance status, N (%)	
0	15 (93.8)
1	1 (6.3)
Number of 2nd line hormone therapies, N (%)	
0	2 (12.5)
1	9 (56.3)
2	3 (18.8)
3	2 (12.5)
Race, N (%)	
Caucasian	12 (75)
African-American	4 (25)
Patients with bone metastases, N (%)	3 (18.8)
Patients with RECIST evaluable soft tissue metastases, N (%)	10 (62.5)

C.

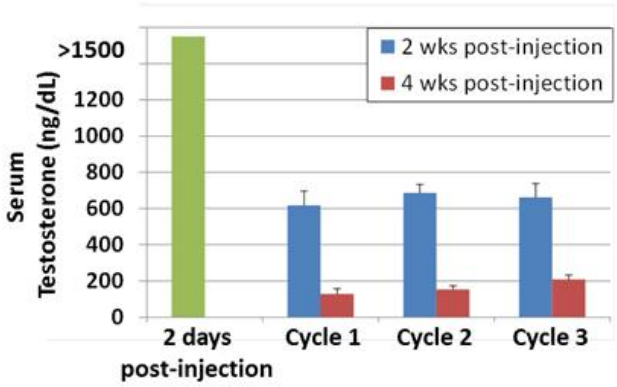


Figure 4. Clinical trial of BAT plus etoposide. (A) Schematic of study design. (B) Baseline characteristics of patients on study. (C) Mean serum testosterone levels at indicated time points for patients on study.

Based on the preclinical results and potential mechanisms for growth inhibition that include androgen-induced double strand breaks and stabilization of AR preventing relicensing, we have conducted a pilot study evaluating the efficacy and safety of pharmacologic doses of testosterone to produce supraphysiologic T levels in conjunction with oral etoposide in chronically castrated men with rising PSA and CRPC, Figure 4a. Patients who had been continuously castrate for more than one year with minimal metastatic disease burden (≤ 5 total bone metastases and ≤ 10 total sites of metastases) and/or rising PSA were eligible, Figure 4b. To achieve rapid cycling between supraphysiologic and near castrate serum T (i.e. BAT) patients received intramuscular injection of 400 mg testosterone cypionate every 28 days. For the first 3 cycles of therapy patients received BAT plus oral etoposide 100 mg po/day days 1-14 of a 28 day cycle. After 3 cycles PSA response and objective response were assessed. Those patients with a PSA that was declining from peak levels and no objective evidence of disease progression or worsening pain were continued on therapy. Given the toxicity associated with etoposide and the lack of clinical response in an earlier trial (39), patients who responding after 3 cycles of testosterone plus etoposide were continued on testosterone alone based on protocol amendment.

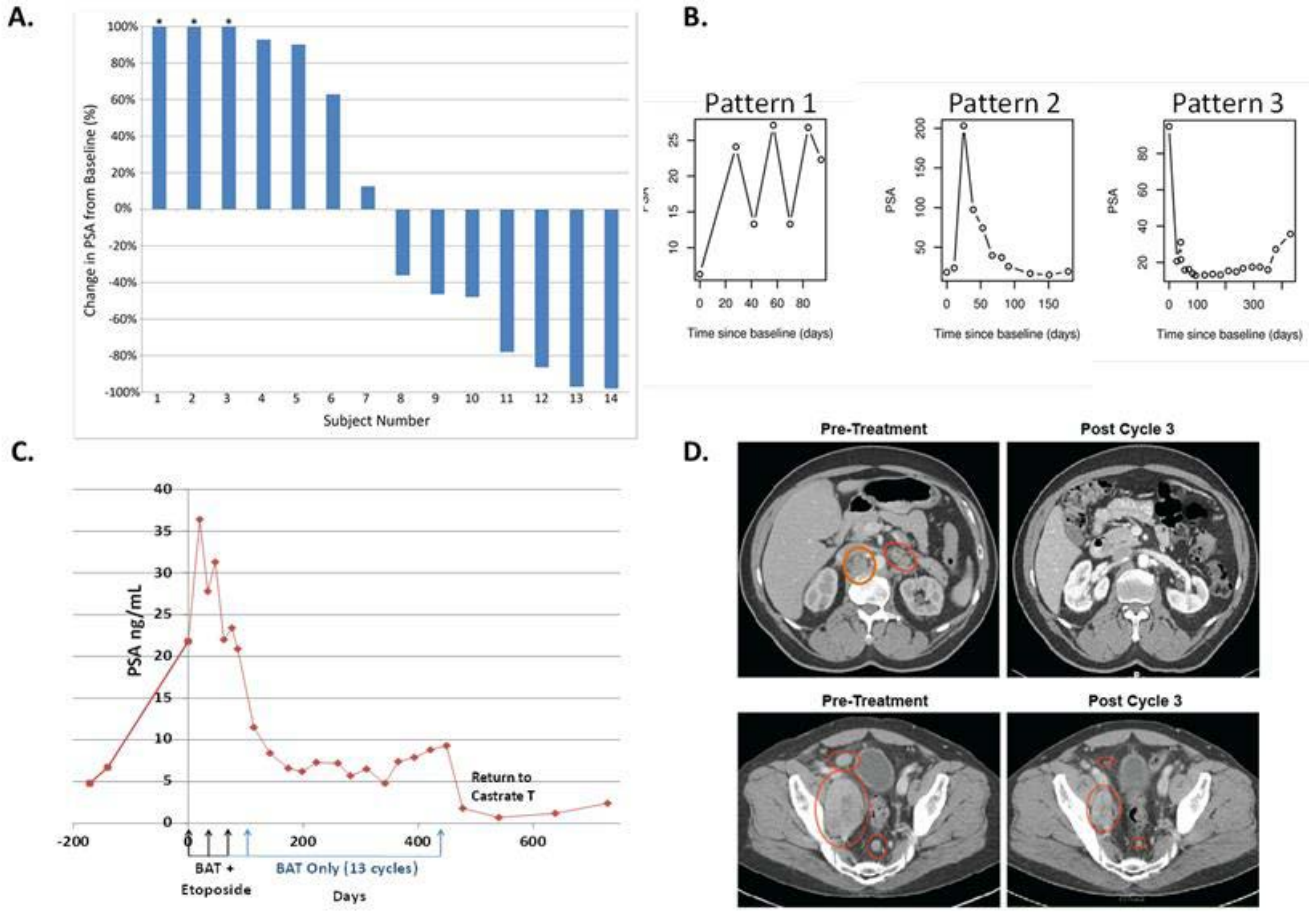


Figure 5. Results from the pilot BAT study in men with CRPC. (A) Waterfall plot of best PSA response with 7/14 men and 4/14 men achieving PSA declines >30% and >50%, respectively (B) Representative patterns of PSA response in pilot study PSA and serum T level in non-responder; (C) PSA response in patient receiving 3 cycles of T+ E and then 13 additional cycles of T alone. Patient demonstrated renewed sensitivity to ADT after progressing on BAT; (D) Objective lymph node complete and partial response after 3 cycles of BAT + E.

Seven of fourteen patients had a decline in PSA from baseline value, Figure 5a. An eighth patient progressing after testosterone treatment for 6 months had a decline in PSA upon reaching castrate level testosterone. Non-responders came off of trial after 3 cycles due to PSA progression. Overall three patterns of PSA response were observed, Figure 5b. For the seven patients that had a PSA decline, the median time to PSA progression was 221 days (range, 95 to 454 days).

The dose of 400 mg T cypionate produces supraphysiologic levels > 1500 ng/dl within 2 days post injection, Figure 4c. At baseline, ten subjects had RECIST-evaluable soft tissue metastases, Figure 4b. Of these patients, two (20%) had progressive disease (PD), three (30%) had stable disease after a median follow up of 91 days (range, 87 to 92 days), four (40%) had partial responses (PRs) and one (10%) had a complete response (CR), Figure 5d. None of the 14 patients completing 3 months of therapy developed new bone metastases. One patient with >50% decrease in PSA had intensification of an isolated tibial metastases on bone scan and was removed from study despite decline in PSA levels. No other patient developed worsening pain on study.

Adverse Events: The majority of adverse events (AEs) occurred during the initial phase of treatment and were largely consistent with known side effects of etoposide. Initial-phase side effects were mostly low grade (i.e. ≤grade 2) and included: nausea (N=10), fatigue (N=9), alopecia (N=9), edema (N=8) and neutropenia (N=3), table 2. Two patients had grade 3 asymptomatic, subsegmental pulmonary embolism. Two subjects did not complete the initial treatment phase, one individual was taken off study after developing grade 2 priapism and a second individual expired due to pneumonia/neutropenic sepsis. AEs occurring during the BAT monotherapy phase of the trial were rare and low grade. Only four subjects experienced an AE during this phase, and all but three AEs were grade 1. Grade 2 events included alopecia and an elevated creatinine in one subject and grade 2 nausea in a separate subject.

Table 2: Adverse events occurring in >15% of subjects and severe (grade ≥4) events.

Adverse Event	Grade 1-2 N (%)	Grade 3-4 N (%)	Any Grade N(%)
Anemia	3 (18.8)	0	3 (18.8)
Dysgeusia	3 (18.8)	0	3 (18.8)
Weight gain	3 (18.8)	0	3 (18.8)
Anorexia	4 (25)	0	4 (25)
Breast sensitivity	4 (25)	0	4 (25)
Neutropenia	3 (18.8)	1 (6.3)	4 (25)
Edema	8 (50)	0	8 (50)
Alopecia	9 (56.3)	0	9 (56.3)
Fatigue	9 (56.3)	0	9 (56.3)
Nausea	10 (62.5)	0	10 (62.5)
Pulmonary embolism	0	2 (12.5)	2 (12.5)
Death	0	1 (6.3)	1 (6.3)

Note: all of those events listed occurred during the testosterone plus etoposide phase of the trial.

None of the 14 patients developed new pain, skeletal events or urinary obstruction due to prostate cancer. Although quality of life was not formally evaluated in the study, most subjects reported enhanced well-being and increased functional activity. Patients with intact sexual function prior to ADT had return of sexual function and libido on BAT.

PSA Reductions to Subsequent Hormonal Therapies: As part of the aforementioned pilot study, we performed a post hoc exploratory analysis on the effect of BAT on subsequent hormonal therapies. Overall, 12 out of 13 subjects had PSA decline to AR-directed therapy post-BAT (one patient continued to show PSA progression upon return to castrate T levels and proceeded to receive docetaxel, the 14th patient remains on BAT (table3). T levels were allowed to return to the castrate range prior to having a second line AR-directed therapy initiated (e.g. abiraterone, enzalutamide, bicalutamide) in 12 patients. Of these 12 subjects, 9 (75%) had a PSA decline below their post-BAT PSA. Of the 6 PSA responders that came off study, 4 (66.7%) had a PSA decline below their post-BAT PSA upon becoming castrate again. All of the patients had received at least one anti-androgen prior to starting the study (Table 3).

Table 3. PSA response to secondary hormonal therapy after return to castrate T-levels post-BAT.

Subject number[a]	Maximum PSA decline upon re-castration (%)[b]	Secondary HT received pre-study	Secondary HT received post-study	Maximum PSA decline upon secondary HT initiation (%)[c]
1	NA	nilutamide, bicalutamide	nilutamide	-44.3
2	-88.2	bicalutamide	none	NA
3	-57.4	bicalutamide	abiraterone	-94.2
4	No decline	bicalutamide	abiraterone	-92.7
5	-52.0	bicalutamide, ketoconazole	enzalutamide	-30.4
6	-58.2	bicalutamide	bicalutamide	-30.8
7	-73.2	bicalutamide	abiraterone	-88.1
8	No decline	bicalutamide, nilutamide	none	NA
9	-92.5	bicalutamide	none	NA
10	No decline	bicalutamide	abiraterone	-63.8
14	-38.2	nilutamide, bicalutamide, abiraterone	enzalutamide	-99.5
15	-35.0	abiraterone	enzalutamide	-78.3
16	-68.4	nilutamide, abiraterone, enzalutamide	enzalutamide	-53.2

After return to castrate T levels post-BAT, ten of 10 (100%) patients receiving second line therapy with either abiraterone (n=4/4) or an anti-androgen [enzalutamide (n=4/4), bicalutamide (n=1/1), nilutamide (n=1/1)] had a PSA decline (range 30.8-99.5%) (Figure 5). Four of 4 patients receiving abiraterone and 3/4 patients on enzalutamide had >50% PSA decline. Of note, two subjects were re-challenged with a first generation anti-androgen (i.e. nilutamide, bicalutamide) and one with enzalutamide after having previously progressed on these agents. These subjects achieved a 44.3%, 30.9% and 53.2% PSA decline upon initiation of nilutamide, bicalutamide and enzalutamide, respectively. The patient re-challenged with enzalutamide had also previously progressed on abiraterone prior to enrolling in this study.

The lessons learned from this pilot trial were that supraphysiologic testosterone could be administered safely to men with metastatic CRPC without producing worsening signs or symptoms due to prostate cancer. While formal testing was not performed, most men reported improved quality of life with increased energy, less fatigue, increased libido and resumption of erectile function in those men with preserved function prior to castrating therapy. PSA decline/response and objective responses were observed in 50% patients who completed three cycles of therapy. While PSA declines were observed, many patients with supposedly CRPC had an initial spike in PSA following the first injection of testosterone. These results suggest that, although these patients demonstrated progressive disease while on chronic castrating therapies as a requirement for enrollment in the trial, the CRPC cells must have continued expression of a functional AR axis as evidenced by the increased expression of an androgen responsive gene, PSA, in response to androgen replacement. Finally, 100% of patients demonstrated PSA response to androgen ablative therapies post-BAT suggesting exposure to BAT has the potential to reverse resistance and re-sensitize CRPC cells to androgen ablative therapies such as abiraterone and enzalutamide.

2. STUDY OBJECTIVES

2.1. Primary Objectives

The primary objective of the study is to determine if treatment with supraphysiologic testosterone (BAT) will improve progression free survival compared to enzalutamide in asymptomatic men with evidence of progressive metastatic CRPC post-treatment with abiraterone.

Progression free survival is defined as the time from randomization to the development of either clinical or radiographic progression as defined below while on therapy with either BAT or enzalutamide.

2.1.1 Clinical Progression

Patients who do not meet the criteria of radiographic progression who are removed from study for worsening symptoms that are attributable to prostate cancer progression will be considered to have clinical progression.

Time to clinical progression will be defined as the time from randomization to documentation in the CRF of any of the following (whichever occurs earlier)

- 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 palliative radiation.

- Development of a skeletal-related event (SRE): pathologic fracture, spinal cord compression, or need for surgical intervention or radiation therapy to the bone.

- Development of clinically significant symptoms due to loco-regional tumor progression (e.g. urinary obstruction) requiring surgical intervention or radiation therapy.

Patients who experience worsening pain after the first cycle of BAT or enzalutamide will be considered to have tumor flare. Patients who are removed from study for pain after first cycle of BAT or enzalutamide will not be considered as having clinical progression and will be considered non-evaluable.

2.1.2 Radiographic Progression

Radiographic progression-free survival is based on parameters suggested by PCWG2 (Appendix 7) and modified RECIST (Appendix 1) as follows:

- On CT scan, radiographic progression will be defined by RECIST criteria (i.e. >20% increase in the sum of target lesions).

- On bone scan, radiographic progression will be defined by PCWG2 criteria as ≥ 2 new bone lesions (Appendix 7). However, for the first reassessment scan only, patients should remain on study 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 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 (Appendix 7).

- Death from any cause will also be considered as progression

2.2. Secondary Objectives

- Further investigate the safety of cyclical parenteral testosterone in asymptomatic men with recurrent castrate resistant prostate cancer. Safety will be evaluated by the incidence, severity, duration, causality, seriousness, and type(s) of adverse events as assessed by the revised National Cancer Institute Common Toxicity Criteria (NCI CTC), version 4.0 published 28 May 2009 (Appendix 2).
- PSA response rate [Proportion of patients achieving a PSA decline ≥ 50% according to Prostate Cancer Working Group (PCWG2) criteria]
- Objective response rate in patients with measurable disease on CT scan using RECIST criteria
- Time to PSA progression to each arm of therapy based on PCWG2 criteria
- Time to Radiographic progression to each arm based on RECIST 1.1 and PCWG2 criteria
- PSA response rate to enzalutamide post-BAT
- PSA response rate to BAT post-enzalutamide
- Overall Survival
- PFS2 (Time from initiation of therapy to progression on crossover treatment)
- Comparison of effect of each treatment arm on quality of life as assessed by patient completion of validated instruments (Appendix 8)

3. PATIENT POPULATION AND SELECTION

Eligible patients will have with metastatic CRPC with no disease related symptoms who have been treated with continuous ADT and have progressed after treatment with abiraterone. Patients will continue on ADT with LHRH agonist (i.e. Zoladex, Trelstar, Eligard or Lupron) or LHRH antagonist (Degarelix) if not surgically castrated throughout the duration of the study to inhibit endogenous testosterone production. Patients will be randomized 1:1 and stratified based on duration of abiraterone acetate therapy (less than or greater than 6 months). A total of 194 patients (97 per treatment arm) will be recruited across 20 treatment sites over a 24 month period.

3.1. Inclusion Criteria

Patients must meet the following criteria to be enrolled in this study:

1. ECOG Performance status ≤2.
2. Age ≥18 years.
3. Histologically-confirmed adenocarcinoma of the prostate.

4. Treated with continuous androgen ablative therapy (either surgical castration or LHRH agonist/antagonist).
5. Documented castrate level of serum testosterone (<50 ng/dl).
6. Metastatic disease radiographically documented by CT/MRI or bone scan.
7. Must have had disease progression while on abiraterone acetate alone or abiraterone acetate in combination with other investigational agents based on:
    - PSA progression defined as an increase in PSA, as determined by 2 separate measurements taken at least 1 week apart

      And/ Or
    - Radiographic disease progression, based on RECIST 1.1 in patients with measurable soft tissue lesions or PCWG2 for patients with bone disease
8. Screening PSA must be ≥ 1.0 ng/mL.
9. Prior treatment with additional second line hormone therapies is permitted.
10. No prior treatment with enzalutamide, ARN-509, ODM-201, galeterone or other investigational AR targeted treatment is allowed.
11. Prior docetaxel for hormone-sensitive prostate cancer is permitted if ≤ 6 doses were given in conjunction with first-line androgen deprivation therapy and >12 months since last dose of docetaxel.
12. Prior treatment with Provenge vaccine and ^{223}Radium (Xofigo) is allowed if >4 weeks from last dose.
13. Patients must be withdrawn from abiraterone for ≥ 2 weeks.
14. Patients must be weaned off prednisone and be off therapy for ≥ 1 week prior to starting therapy.
15. Acceptable liver function:
    - Bilirubin < 2.5 times institutional upper limit of normal (ULN)
    - AST (SGOT) and ALT (SGPT) < 2.5 times ULN
16. Acceptable renal function:
    - Serum creatinine < 2.5 times ULN
17. Acceptable hematologic status:
    - Absolute neutrophil count (ANC) ≥ 1500 cells/mm^3 (1.5 ×10^9/L)
    - Platelet count ≥ 100,000 platelet/mm^3 (100 ×10^9/L)
    - Hemoglobin ≥ 9 g/dL
18. At least 4 weeks since prior radiation.
19. Ability to understand and willingness to sign a written informed consent document.
20. Patients on either treatment arm will be considered for crossover if they demonstrate evidence of radiographic disease progression from the initial treatment.

3.2. Exclusion Criteria

1. Pain due to metastatic prostate cancer requiring treatment intervention.
2. ECOG Performance Status ≥ 3.
3. Prior treatment with enzalutamide is prohibited
4. Prior treatment with docetaxel or cabazitaxel for metastatic castration-resistant prostate cancer is prohibited.

5. Requires urinary catheterization for voiding due to obstruction secondary to prostatic enlargement well documented to be due to prostate cancer or benign prostatic hyperplasia (BPH).
6. 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, severe and extensive spinal metastases with concern over spinal cord compression, extensive liver metastases)
7. 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
8. Active uncontrolled infection, including known history of HIV/AIDS or hepatitis B or C.
9. Any psychological, familial, sociological, or geographical condition that could potentially interfere with compliance with the study protocol and follow-up schedule.
10. Patients receiving anticoagulation therapy with Coumadin are not eligible for study. [Patients on non-coumadin anticoagulants (Lovenox, Xarelto, etc.) are eligible for study. Patients on Coumadin who can be transitioned to lovenox prior to starting study treatments will be eligible]
11. Patients with prior history of a thromboembolic event within the last 12 months that is not being treated with systemic anticoagulation are excluded.
12. Patients allergic to sesame seed oil or cottonseed oil are excluded.
13. Major surgery (eg, requiring general anesthesia) within 3 weeks before screening, or has not fully recovered from prior surgery (ie, unhealed wound). Note: subjects with planned surgical procedures to be conducted under local anesthesia may participate.

3.3 Inclusion of women and minorities

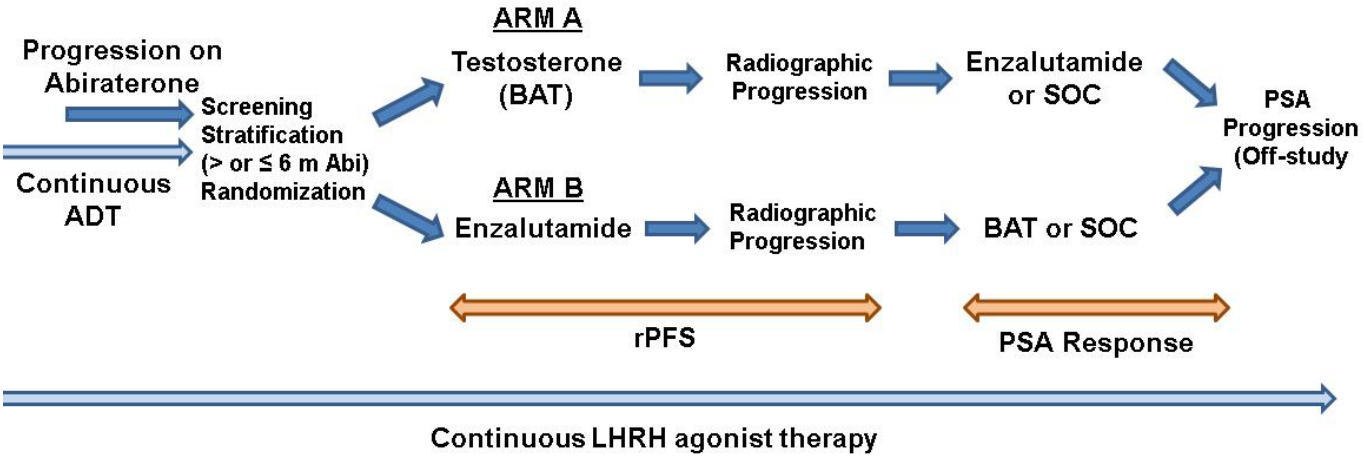
This study is focused on prostate cancer, therefore is applicable to men only. Women and children will not be included on this study. Men from all ethnic and race groups are eligible for this study**.**

4. TREATMENT PLAN

4.1. Study Design

Men with metastatic CRPC with no disease related symptoms who have been treated with continuous ADT and have progressed after treatment with abiraterone (pre- chemotherapy for metastatic disease) will be treated on a randomized, multi-Institutional open label study to determine if treatment with intramuscular T, given on a dose/schedule designed to achieve serum T levels that rapidly cycles from the polar extremes of supraphysiologic to near castrate levels [i.e. termed Bipolar Androgen Therapy (BAT)], will improve progression free survival, quality of life and metabolic syndrome vs. enzalutamide as standard therapy.

Study Design Schematic



4.2. Study Treatments and Treatment Scheme

4.2.1. Study Treatments

Patients on BAT (Arm A) will receive testosterone cypionate or testosterone enanthate administered as an intramuscular injection. These two forms of testosterone exhibit identical pharmacokinetic properties and may be used interchangeably in this study based on availability at individual study sites. These agents are administered as intramuscular injections given deep in the gluteal muscle. They should not be given intravenously. Testosterone cypionate is in cottonseed oil containing benzyl alcohol as a preservative. Testosterone enanthate is in sesame oil with chlorobutanol as a preservative. A dose of 400 mg of either agent will be injected intramuscularly (IM) every 28 days. This dose was selected based on data demonstrating that it produces an initial supraphysiologic serum level of T (i.e. > 1500 ng/dL or 3-10 times normal level) with eugonadal levels achieved at the end of two weeks and near castrate levels after 28 days.

Patients randomized to enzalutamide (Arm B) will be prescribed enzalutamide 40 mg tablets and instructed to take 4 tablets per day orally for 28 days/cycle.

4.2.2. Treatment Scheme

Patients will continue on ADT with LHRH agonist (i.e. Zoladex, Trelstar, Eligard or Lupron) or LHRH antagonist (Degarelix) if not surgically castrated throughout the duration of the study to inhibit endogenous testosterone production. Patients will be randomized 1:1 and stratified based on duration of abiraterone acetate therapy (less than or greater than 6 months). Patients randomized to BAT will receive 400 mg T-cypionate or T-enanthate IM every 28 days. Patients randomized to enzalutamide will receive daily oral dose of 160 mg for 28 days/cycle. Each cycle is 28 days in length.

Patients will have PSA level checked every cycle. Every 3 cycles patients will have repeat bone/CT scans to evaluate treatment response. Patients will remain on treatment until evidence of disease progression on CT scan (per RECIST criteria) or with progressive bone metastatic disease (per the PCWG2 definition) or symptomatic progression due to prostate cancer. Patients with PSA progression but with disease response or stable disease on imaging studies will remain on study.

Patients with radiographic disease progression on either arm will be given opportunity to cross-over to the opposite treatment. Patients who opt to cross-over must wait 4-6 weeks from time patient has confirmed radiographic disease progression before starting cross-over treatment. Patients on the BAT arm A with confirmed radiographic disease progression can cross over to receive enzalutamide beginning 4-6 weeks after time of confirmed radiographic progression. Patients on the enzalutamide arm B will be allowed to cross-over to receive BAT beginning 4-6 weeks after time of confirmed radiographic progression. Patients who opt to cross over will remain on study and continue respective therapies for at least 3 cycles. Patients will have PSA checked each cycle (i.e. every 4 weeks) and repeat bone and CT scans every 3 cycles. Patients demonstrating PSA response after 3 cycles will remain on study until evidence of PSA progression per PCWG2 criteria or confirmed radiographic disease progression.

Patients with clinical progression due to prostate cancer who meet study exclusion criteria (section 3.2) will be permitted to cross-over to the opposite treatment. Patients with clinical progression due to pain from prostate cancer are not permitted to cross-over.

Patients who opt not to cross-over will complete end of study visit and will be removed from study.

4.3. Dosing Delays and Modifications

Treatment will be given on indicated days ± 5 days. Patients who develop seizures, pulmonary embolus or other thromboembolic event will be removed from study.

There will be no dose modifications for testosterone in this study.

Dose modification for enzalutamide will be according to the institution's standard of care practice. Enzalutamide Dose reduction to label recommended dose of 80 mg/day is allowed for patients on concomitant CYP2C8 inhibitors (gemfibrozil) or strong CYP3A4 inducers. Dose reduction is at the discretion of the treating physician.

Patients may temporarily suspend study treatment if they experience toxicity that is considered to be related to study drugs and requires a dose to be held. Treatment delay will be allowed up to 4 weeks due to drug related toxicities.

If, in the judgment of the investigator, the patient is likely to derive clinical benefit from resuming the study drugs after 4 weeks, the study drug may be restarted with the approval of the Medical Monitor (Dr. Samuel Denmeade).

Dose interruptions for reason(s) other than toxicity, such as surgical procedures, may be allowed with

Medical Monitor approval. The acceptable length of interruption will depend on agreement between the investigator and the Medical Monitor.

4.4. Removal of Patients from Study

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

1. As defined by the protocol, evidence of disease progression based on radiographic progression or worsening symptoms.
2. Unacceptable adverse event(s), including:
   - Patients develop new or worsening pain deemed by the investigator to be due to disease progression
   - Patients develop urinary outlet obstruction well documented and 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 due to prostate cancer progression or drug toxicity.
   - Patents develop hypersensitivity or anaphylactoid reactions to testosterone injection.
   - Any evidence of severe dose limiting toxicities secondary to treatment with enzalutamide that cannot be controlled with standard therapy (e.g. nausea/vomiting not controlled with oral antiemetic regimen).
3. Intercurrent illness that prevents further participation.
4. Experiencing a treatment delay of longer than 4 weeks due to drug toxicity; however, if the patient is receiving clinical benefit, treatment may be delayed for longer than 4 weeks and then resumed at the discretion of the Investigator and the approval of the Medical Monitor (Dr. Samuel Denmeade).
5. Patient refuses further treatment through the study and/or withdraws consent to participate
6. Patient is noncompliant with respect to taking drugs, keeping appointments, or having tests required for the evaluation of drug safety and efficacy.
7. 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.
8. Under no circumstance will care of a withdrawn patient be adversely affected by a decision to withdraw or be withdrawn from the study.
9. Deterioration in ECOG performance status to grade 3 or higher.

4.5 Criteria for Discontinuation of Study Treatment due to Development of Clinical Progression due to Prostate Cancer

Due to the nature of the treatment, there is concern that patients could experience potential development of prostate cancer related pain due to BAT. 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 progression free survival. Patients that have unequivocal

clinical progression without radiographic progression who continue to meet study exclusion criteria (section 3.2) will be permitted to cross-over to the opposite treatment. Patients who do not meet these original exclusion criteria and patients with clinical progression due to pain from prostate cancer are not permitted to cross-over and should be removed from the study and evaluated for standard of care therapy. 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 palliative radiation.
2. Development of a skeletal-related event (SRE): pathologic fracture, spinal cord compression, or need for surgical intervention or radiation therapy to the bone.
3. Development of clinically significant symptoms due to loco-regional tumor progression (e.g. urinary obstruction) requiring surgical intervention or radiation therapy.

All patients meeting the criteria for unequivocal clinical progression should have repeat imaging studies (i.e. bone and CT scans) if not done within the past month of meeting said criteria.

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. 1.2014) (Appendix 3) 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.

4.7 Concomitant Therapy

The use of any concurrent medication from screening and while on study, prescription or over-the-counter, is to be recorded on the patient's CRF along with the reason the medication was taken. In addition, tobacco and alcohol use will be collected.

Concurrent use of another clinical investigational drug or device while on study is prohibited.
Supportive care medications are permitted with their use following institutional guidelines. For patients who did not undergo orchiectomy, concurrent treatment with LHRH analogue is mandatory and must be recorded.
The following supportive care medications are considered permissible during the study:

- Conventional multivitamins, selenium and soy supplements
- Systemic glucocorticoid administration such as “stress dose” glucocorticoid up to maximum of 4 mg/day dexamethasone or dexamethasone equivalent is permitted if clinically indicated. Selection of corticosteroid, dose and duration is at the discretion of the treating physician.

- Dutasteride or finasteride if being used to treat BPH and only if patients are on the medication for at least 3 months prior to Study Day 1
- Bisphosphonate and denosumab usage is allowed only if patients are on the medication for at least 3 months prior to Study Day 1
- Transfusions and hematopoietic growth factors per institutional practice guidelines

4.8 Prohibited Concomitant Medications

Concomitant therapy during the treatment phase of the study with any of the following listed is prohibited:

- Chemotherapy
- Immunotherapy
- Bicalutamide, nilutamide, flutamide
- Systemic ketoconazole (or other azole drugs such as fluconazole and itraconazole)
- Diethylstilbestrol, PC-SPES, and other preparations such as saw palmetto thought to have endocrine effects on prostate cancer
- Radiopharmaceuticals such as xofigo (223Ra), strontium (89Sr) or samarium (153Sm)
- Other experimental drugs or treatments

5 STUDY ACTIVITIES

5.1 Screening Period

All patients must sign a written informed consent form before study specific screening procedures are performed. Screening procedures to evaluate patient eligibility for the study will be conducted within 28 days prior to Cycle 1 Day 1. Prior to enrollment, patient must have documented insurance coverage demonstrating ability to pay for enzalutamide in event patient is randomized to this treatment arm. If the patient meets eligibility and screening requirements he will be randomized and will return to the site for the Cycle 1 Day 1 visit and dosing. All required treatment and post-treatment study procedures and assessments must be done within 5 days (+/-) of the specified study visit date.

Initial Registration Process:
Eligible patients will be registered on study centrally at the Johns Hopkins Sidney Kimmel Comprehensive Cancer Center by the TRANSFORMER Study Manager.

To register a patient, the following documents must be completed and emailed to the Study Manager, Haiyi (Harry) Cao, at hcao7@jhmi.edu:

- Signed/dated patient consent form
- Eligibility checklist
- Copies of the prostate cancer pathology report
- Copies of pre hormone therapy/chemotherapy or radiation therapy
- Screening labs
- CT and bone scan reports

- Other materials may also be sent if considered pertinent for confirming patient eligibility

The Study Manager will review the documents to confirm eligibility. To complete the registration process the Study Manager will:

- Review the eligibility checklist and the related source documents
- Patients found to be ineligible for participation after being consented will be considered screen failures, and documented as such in the Screening and Enrollment Log
- Once eligibility is confirmed, patients will be randomized to a treatment group according to the randomization schedule. Patients will be randomized to receive either BAT or enzalutamide in a 1:1 ratio. A randomization assignment will be emailed to sites with confirmation of registration

All patients must commence treatment within 5 calendar days of randomization.

5.2 Randomization

Once eligibility is confirmed, patients will be randomized to a treatment group according to the randomization schedule. Patients will be randomized to receive either BAT or enzalutamide in a 1:1 ratio. All patients must commence treatment within 5 calendar days of randomization.

5.3 Stratification

In this study patients will be stratified according to duration of response to prior therapy with abiraterone (i.e. < 6 months or ≥ 6 months response).

5.4 Screening Studies (performed within 28 days before Cycle 1 Day 1)

1. Comprehensive medical history and physical exam, including height and weight, and medications.
2. ECOG Performance status (PS)
3. Body Composition (Body weight, body mass index)
4. CBC (Complete blood count) with differential and platelet count
5. CMP (Comprehensive Metabolic Panel - Sodium, Potassium, Chloride, BUN, Serum Creatinine, Calcium, Total Protein, Albumin, Total Bilirubin, AST, ALT, Alkaline Phosphatase, HCO_3)
6. Serum PSA, testosterone
7. Lipid Panel (fasting)
8. Androgen Panel [dihydrotestosterone (DHT), free testosterone, estradiol, dehydroepiandostenedione sulfate (DHEA-S), DHEA and sex hormone binding globulin (SHBG)]
9. Metabolic Panel (fasting glucose, hemoglobin A1c, fasting insulin, serum C-telopeptide, osteocalcin)
10. C Reactive Protein
11. Baseline EKG
12. Partial Prothrombin (PTT) and Prothrombin Times (PT)
13. Staging imaging with CT and bone scintigraphy (bone scan)

14. Blood drawn for to assess level of full length and androgen receptor variant 7in circulating tumor cells (CTC-ARV7) (Appendix 4)
15. Blood drawn for to evaluate presence of AR mutation in circulating tumor DNA (AR-DNA) (Appendix 4)
16. Quality of life surveys (FACIT-Fatigue Scale, RANDSF-36, IIEF, Brief Pain Inventory, International Positive and Negative Affect Schedule Short Form (I-PANAS-SF) (mood assessment) (Appendix 8)

5.5 Treatment Period

All required treatment and post-treatment study procedures and assessments must be done within 5 days (+/-) of the specified study visit date.

1. For BAT Arm A patients will have a clinic visits every cycle to receive testosterone injection and undergo assessment of toxicity, ECOG performance status and vital signs
2. For enzalutamide Arm B patients will have a clinic visit every 3 cycle to receive new drug prescription, undergo assessment of toxicity, ECOG performance status and vital signs
3. PSA every cycle for both Arms. PSA may be performed at outside laboratory for patients randomized to Arm B as long as patient can have study done at the same outside lab each time. (PSA is not performed at Cycle 1 Day 1)
4. CBC, Comp Panel, testosterone, QOL surveys after first cycle for both arms (V3)
5. CBC, Comp Panel, Lipid Panel, Metabolic Panel, CRP after every 3 cycles for both arms (V5, V8, V11, etc.)
6. Androgen Panel after first 3 cycles and at progression
7. CT scan, bone scan every 3 months for both arms (V5, V8, V11, etc.)
8. Blood drawn for research testing to assess level of full length and variant androgen receptor and circulating DNA for AR mutation at V5 (Appendix 4)
9. Quality of life surveys after first cycle (V3) and then every 3 months for both arms (V5, V8, V11, etc.)

5.6 End of Initial Treatment (at the time of progression)

At the time of progression, the following assessments will be performed:

1. Physical examination
2. Vital signs
3. ECOG
4. Concomitant medication
5. Adverse events
6. Comprehensive metabolic panel, CBC, and serum PSA
7. Quality of life surveys (FACIT-Fatigue Scale, RANDSF-36, IIEF, Brief Pain Inventory, International Positive and Negative Affect Schedule Short Form (I-PANAS-SF) (mood assessment) (Appendix 8)
8. Patients must also have blood drawn for CTCs to look for changes in AR-V7 and total AR expression. The CTCs samples will be either collected at the end of study visit for patients who opt not to cross-over, or at the cross over visit for patient who opt to cross over to receive the opposite

treatments (CTCs samples will be collected before the start of the opposite therapy for cross over patients)

9. Patients must additional have labs drawn for Lipid Panel, Androgen Panel [dihydrotestosterone (DHT), free testosterone, estradiol, dehydroepiandostenedione sulfate (DHEA-S), DHEA and sex hormone binding globulin (SHBG)] and Metabolic Panel [fasting glucose, hemoglobin A1c, fasting insulin, serum C-telopeptide, osteocalcin]

Patients who demonstrate radiographic progression on CT or bone scan will have the option to cross-over to opposite arm of therapy. Patients with clinical progression due to prostate cancer who continue to meet study exclusion criteria (section 3.2) will be permitted to cross-over to the opposite treatment. Patients with clinical progression due to pain from prostate cancer are not permitted to cross-over.

Patients who are not eligible or who opt not to cross-over will complete end of study visit and will be removed from study.

Please refer to the study calendar for assessments to be performed at End of Treatment/Progression Visit.

5.7 Crossover Treatment Period

Patients with radiographic disease progression or these with clinical progression who continue to meet exclusion eligibility criteria (section 3.2) on either arm will be given opportunity to cross-over to the opposite treatment. Patients who opt to cross-over must wait 4-6 weeks from time patient has confirmed radiographic disease progression before starting cross-over treatment. Patients on the BAT arm A with confirmed radiographic disease progression can cross over to receive enzalutamide beginning at least 2 and no more than 6 weeks after time of confirmed radiographic progression. Patients on the enzalutamide arm B will be allowed to cross-over to receive BAT beginning at least 2 and no more than 6 weeks after time of confirmed radiographic progression. Patients who opt to cross over will remain on study and continue respective therapies for at least 3 cycles. Patients will have PSA checked each cycle (i.e. every 4 weeks) and repeat bone and CT scans every 3 cycles. Patients demonstrating PSA response after 3 cycles will remain on study until evidence of PSA progression per PCWG2 criteria or confirmed radiographic disease progression.

5.8 End of Study Visit (30-day safety follow-up)

After the initial treatment arm, for those who choose to not cross-over and come off study treatment, they will come for an end of study visit within 30 days from their confirmed radiographic progression on CT or bone scan.

For those patients who crossed over to receive the opposite arm of therapy, they will come for an end of study visit within 30 days from their second disease progression (evidence of PSA progression per PCWG2 criteria or confirmed radiographic disease progression.

For patients with clinical progression that requires patient to come off study (i.e. worsening pain, obstructive symptoms) end of study visit must be scheduled within 30 days of symptoms.
The following assessment will take place at the End of Study visit:

1. Physical examination
2. Vital signs
3. ECOG
4. Concomitant medication
5. Adverse events
6. Comprehensive metabolic panel, CBC, and serum PSA
7. Quality of life surveys (FACIT-Fatigue Scale, RANDSF-36, IIEF, Brief Pain Inventory, International Positive and Negative Affect Schedule Short Form (I-PANAS-SF) (mood assessment) (Appendix 8)
8. All patients meeting the criteria for unequivocal clinical progression should have repeat imaging studies (i.e. bone and CT scans) if not done within the past month of meeting said criteria.

5.9 Early Discontinuation

For patients who come off the study treatments due to an adverse event or unacceptable toxicity related to the study drugs, they will come back to the clinic for an end of study visit.

If early discontinuation is due to an adverse event or unacceptable toxicity related to 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.

5.10 Survival

For patients who have died, date of death will be collected and overall survival duration will be calculated from randomization to death.

For patients who are still alive, date of last contact (**patients who are lost to follow up do not need to be contacted**) will be collected and overall survival will be censored at this date.

6 STUDY ASSESSMENTS

6.1 Assessing Response in Measurable Disease

In patients with measurable disease, 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 (Appendix 1) and for bone lesions based on PCWG2 criteria.

After 3 cycles of study treatment, patients will remain on study unless they show evidence of clinical or radiographic progression. On CT scan, radiographic progression will be defined by RECIST criteria (i.e. >20% increase in the sum of target lesions). On bone scan, radiographic progression will be defined by PCWG2 criteria as ≥ 2 new bone lesions (Appendix 7). However, for the first reassessment scan only, patients should remain on study and have a confirmatory scan performed 12 weeks later. If this confirmatory scan shows 2 or more additional new lesions, this defines progression. The date of progression is the date of the *first* reassessment bone scan. If the confirmatory scan is 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 (Appendix 7).

6.2 Assessing PSA Response to BAT

PSA is an androgen-regulated gene. Therefore, BAT therapy is likely to produce an initial increase in PSA levels even in patients who will respond to treatment. In the pilot study, we observed PSA increase up to 10-fold after the first injection of testosterone, even in some patients who eventually had decline in PSA below baseline. Therefore to assess PSA response in this study we will use PCWG2 criteria with some modification:

1. Patient with PSA that continues to increase above baseline will be considered to have PSA progression (Figure 6A).
2. Patient with PSA that decreases >50% below pretreatment baseline while on BAT will be considered a PSA responder (Figure 6B).
3. Patients with initial increase in PSA in response to BAT with subsequent decline >50% below baseline will also be considered a PSA responder (Figure 6C).
4. Patients whose PSA increases and then declines from peak levels but does not >50% decrease below baseline will not be considered a PSA responder (Figure 6D).

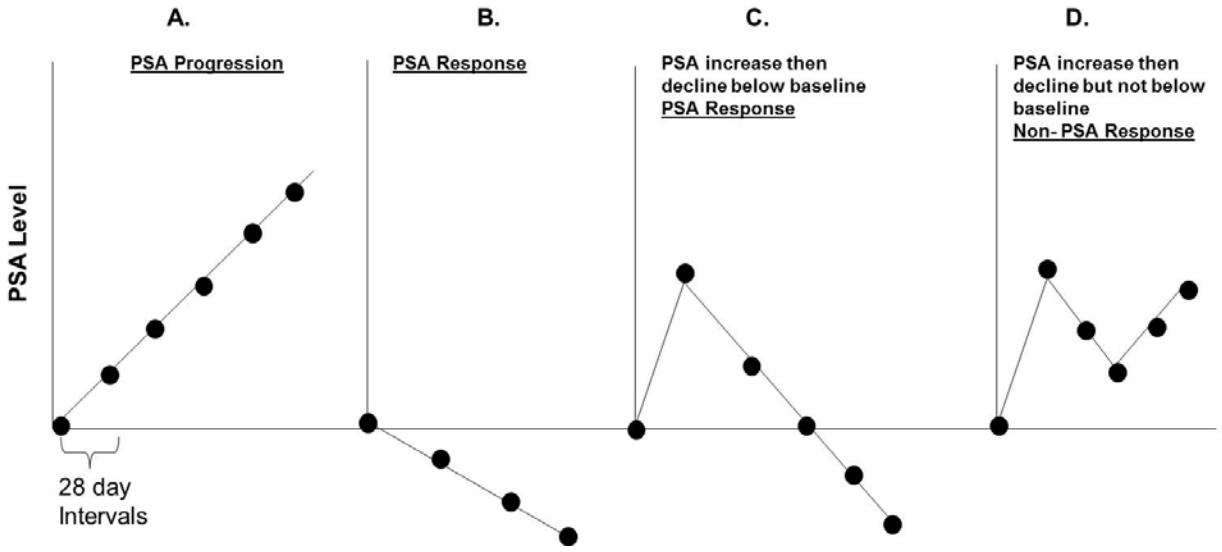


Figure 6. Examples of PSA response patterns on study consistent with PSA progression or response

6.3 Quality of life (QoL) and metabolic studies:

QoL will be assessed using , FACIT-F Version 4, RAND-SF36 Quality of Life Survey, IIEF, Brief Pain Inventory, I-PANAS-SF (Appendix 8). The surveys will be administered at baseline randomization, one month post-randomization, three months post-randomization, six months, 12 months post-randomization and at the time of progression.
For each module, summary statistics of the scores will be reported at baseline randomization, one month post-randomization, three months post-randomization, six months, 12 months post-randomization and at the time of progression.

6.4 Correlative Studies

6.4.1 AR-Variant Studies

To determine levels of full length AR and AR-V7, blood will be obtained from patients at screening, after 3 cycles of therapy on each arm and then at time of radiographic progression. Blood samples are collected and shipped to the Johns Hopkins Laboratory within 24 hrs according to procedures described in Appendix 4.

6.4.2 AR Mutation Studies

An additional documented mechanism of resistance to androgen ablative therapies is mutation of the androgen receptor (AR) that can lead to reactivation particularly if the point mutation is in the ligand-binding domain (LBD) of AR (40). Beltran et al used a whole exome sequencing approach with formalin-fixed paraffin-embedded (FFPE) biopsy tissue and determined that 44% of CRPCs studied harbored genomic alterations involving the AR gene (AR), including AR copy number gain (24% of CRPCs) or AR point mutation (20% of CRPCs) (41). AR mutations can also frequently be detected in CTCs isolated from patients with CRPC. Jiang et al used the Veridex system to isolate CTCs and documented AR mutations in 20/35 (57%) patients (42). Many of these mutations occur in the LBD of AR and can broaden ligand specificity, and some confer resistance by converting the AR antagonist into an agonist of the mutant receptor (43-45). Recently, a specific mutation F876L that may confer response to enzalutamide has been described (46, 47). Joseph et al (48) analyzed circulating tumor DNA (ctDNA) in plasma from 29 patients who participated in the phase I portion of a clinical trial of ARN-509 using the sensitive, emulsion PCR-based BEAMing (Beads, Emulsions, Amplification, and Magnetics) method (49). This study specifically looked for the emergence of the F876L LBD mutation and found it in 3/29 (10%) ARN-509 treated patients (48).

To detect new AR mutations will require sequencing of the entire AR gene that may be present in rare amounts in the circulating tumor DNA pool. To accomplish this task we will utilize a new approach known as the Safe-Sequencing System (Safe-SeqS) developed by our co-investigator Dr. Vogelstein and colleagues (50). This approach is designed to overcome the high error rate seen massive parallel sequencing used to detect rare variants of any specific gene.

To detect the effect of BAT on AR mutation in circulating tumor DNA, blood will be obtained from patients (n=60) in the BAT arm (Arm A) or enzalutamide arm (Arm B) at screening and after 3 cycles of BAT or enzalutamide. Blood samples are collected and shipped to the Johns Hopkins Laboratory within 24 hrs according to procedures described in Appendix 4.

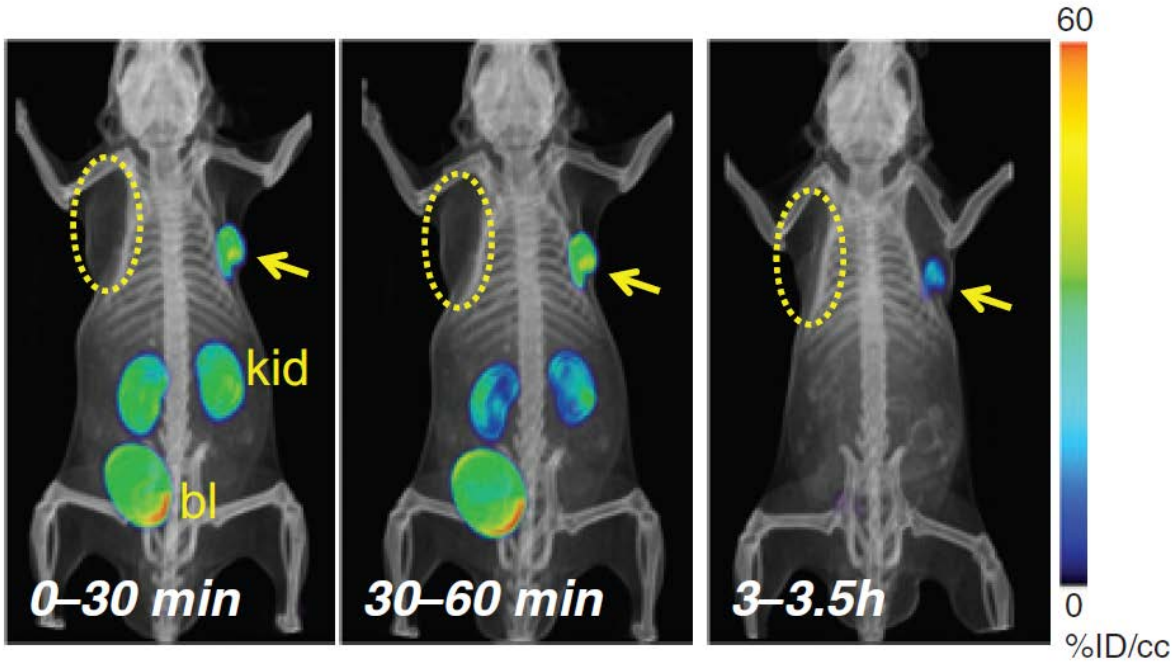
6.4.3 Imaging Studies

6.4.3.1 ^{18}F-DCFPyL PSMA-PET Imaging (Johns Hopkins Only)

PSMA is a type II plasma membrane protein expressed abundantly in prostate cancer epithelia and has been the focus of a number of therapeutic and imaging strategies (51). A potential role for PSMA as a candidate imaging biomarker of AR activity is based on reports showing that androgen suppresses PSMA expression due to the presence of an AR-regulated PSMA enhancer that downregulates PSMA transcription in response to androgens (52).

To improve upon the pharmacokinetics of ^{18}F-DCFBC, a novel second generation low-molecular weight PSMA-targeted PET radiopharmaceutical has been synthesized 2-(3-{1-carboxy-5-[(6-[18F]fluoro-pyridine-3-carbonyl)-amino]-pentyl}-ureido)-pentanedioic acid, (^{18}F-DCFPyL) (54). ^{18}F-DCFPyL displays high affinity for PSMA with a Ki value of 1.1 ± 0.1 nmol/L. In an immunocompromised mouse model ^{18}F-DCFPyL clearly delineated PSMA expressing PC3 PIP prostate tumor xenografts on imaging with PET. At 2 hours post-injection, 39.4±5.4 percent injected dose per gram of tissue (%ID/g) was evident within the PSMA positive PC3 PIP tumor, with a ratio of 358:1 of uptake within PSMA positive PC3 PIP to PSMA negative PC3 flu tumor placed in the opposite flank. At or after 1 hour post-injection, minimal non-target tissue uptake of ^{18}F-DCFPyL was observed. By 3.5 hours after injection, only the PSMA positive tumor was visible with no radiochemical background in liver or the gastrointestinal tract to obscure potential metastases.

Figure below from Chen et al. (54) illustrates PET-CT volume-rendered composite images representing the time course of radiochemical uptake after administration of ^{18}F-DCFPyL. PSMA positive PC3 PIP (arrow) and PSMA negative PC3 flu (dotted oval) tumors are present in subcutaneous tissues posterior to opposite forearms, as indicated. The mouse was injected intravenously with 0.38 mCi (14.1 MBq) ^{18}F-DCFPyL at Time 0. By 30 minutes post-injection radiochemical uptake was evident within the PIP tumor and kidneys. Radioactivity receded from kidneys faster than from tumor, and was not evident within kidneys by 3.5 hours post-injection. Radioactivity within bladder was due to excretion. At no time was radiochemical clearly visualized within the PSMA negative PC3 flu tumor. (kid = kidneys, bl= urinary bladder)



Dosimetry calculations demonstrated a total effective dose of 0.0136 mGy/MBq (50.32 mrem/mCi). The organ with the highest mean absorbed dose per unit administered activity was the urinary bladder wall, 0.15 mGy/MBq (555 mrem/mCi), followed by the kidneys at 0.05 mGy/MBq (185 mrem/mCi). The bladder wall is the dose-limiting organ. On the basis of the dosimetry results a

maximum of 9 mCi (331 MBq) can be administered without exceeding the 50 mGy critical organ dose limit (urinary bladder wall in this case), for a single administration of radioactive material for research use as specified in Code of Federal Regulations 21, part 361. These data demonstrates ^{18}F-DCFPyL as a viable, new PET imaging agent for PSMA-expressing tissues, especially prostate cancer, and warrants further evaluation in translational clinical studies to determine its use for prostate cancer detection.

^{18}F-DCFPyL is currently only available for testing at Johns Hopkins under FDA IND #121064. We propose to use ^{18}F-DCFPyL PET to detect changes in PSMA expression metastatic prostate cancer in response to BAT or enzalutamide. Expression changes are determined by visual qualitative and quantitative SUV analysis. Correlation will be made to sites of suspected metastatic disease detected by standard conventional imaging modalities (CIM) for prostate cancer which includes IV contrast CT of chest/abdomen/pelvis and whole body bone scintigraphy. We expect that patients progressing on ADT and Abiraterone will have relatively low PSMA signal in metastatic sites due to suppression of PSMA expression due to upregulation of AR expression and AR activity despite castrate T levels. Following BAT, we expect to see PSA levels initially increase and PSMA expression decrease due to enhanced AR activity. Following enzalutamide, we expect to see PSA levels initially decrease and PSMA expression increase due to decreased AR activity.

Patients on either arm of the trial at Johns Hopkins will be given opportunity to undergo ^{18}F-DCFPyL-PET imaging (Appendix 5). Patients will sign separate consent for the imaging portion of the study. Imaging will be performed according to standard PET/CT imaging procedures in place at Johns Hopkins, Appendix 5. Patients (n=10/arm) will have ^{18}F-DCFPyL-PET imaging before starting BAT or enzalutamide and then have repeat imaging after 12 weeks of treatment on either arm.

The medical imaging devices (PET-CT) are FDA approved. ^{18}F-DCFPyL (FDA IND #121064) will be used under the auspices of the IND regulations as provided in the Code of Federal Regulations.

7 Requirement for an Independent Research Monitor

For research defined in this protocol which is considered greater than minimal risk, the DOD requires IRB approval, by name, of an independent research monitor with expertise consonant with the nature of risk(s) identified within the research protocol. The monitor for this trial will be Dr. Christine Hann, Assistant Professor of Oncology at Johns Hopkins University School of Medicine.

Research Monitor functions will include:

- Observing recruitment, enrollment and consent procedures
- Overseeing study interventions and interactions,
- Reviewing monitoring plans,
- Overseeing data collection and analysis
- Discussion of the protocol with investigators, interview human subjects

The Research Monitor shall have the authority to stop the research in progress, remove individual human subjects from the protocol and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRBs can assess the monitor's report. The Research Monitor has the responsibility to promptly report observations and findings to the IRB and the DHRPO.

There should be no apparent conflict of interest and the monitor cannot be under the supervision of the overall PI or site PIs or other investigators or research staff involved in the study.

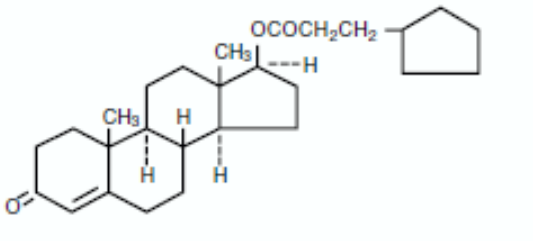
8 Safety Assessment

Safety will 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 CTC version 4.0 published 28 May 2009 to assess the severity of AEs and toxicities (see Appendix 2). All observed or volunteered adverse events regardless of treatment group or causal relationship to study drug will be recorded on the adverse event page(s) of the case report form (CRF).

9 PHARMACEUTICAL INFORMATION-Testosterone Cypionate, Testosterone Enanthate , Enzalutamide

9.1 Testosterone Cypionate Drug Characterization

9.1.1 Drug Name: Testosterone Cypionate (DEPO-Testosterone Injection)



9.1.2 Chemical Name: androst-4-en-3-one,17-(3-cyclopentyl-1-oxopropoxy)-, (17ß)-

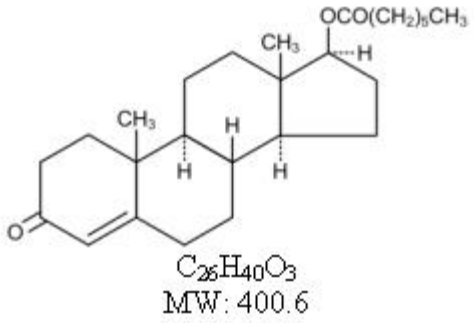
9.1.3 Molecular Formula: $C_{27}H_{40}O_3$ Molecular Weight: 412.61 g/mol

9.1.4 Description

DEPO-Testosterone Injection, for intramuscular injection, contains testosterone cypionate which is the oil-soluble of the androgenic hormone testosterone. Testosterone cypionate is a white or creamy white crystalline powder, odorless or nearly so and stable in air. DEPO-Testosterone Injection is available in two strengths, 100 mg/mL and 200 mg/mL testosterone cypionate.
Each mL of the 100 mg/mL solution contains:
Testosterone cypionate..... 100 mg
Benzyl benzoate ..... 0.1 mL
Cottonseed oil ..... 736 mg
Benzyl alcohol (as preservative) ..... 9.45 mg
Each mL of the 200 mg/mL solution contains:
Testosterone cypionate ..... 200 mg
Benzyl benzoate ..... 0.2 mL
Cottonseed oil ..... 560 mg
Benzyl alcohol (as preservative) ..... 9.45 mg

9.2 Testosterone Enanthate Drug Characterization

9.2.1 Drug Name: Testosterone Enanthate (Delatestryl) Structural formula:



9.2.2 Chemical Name: (androst-4-en-3-one, 17-[(1-oxoheptyl)-oxy]-, (17β)-.

9.2.3 Molecular Formula: $C_{26}H_{40}O_3$ Molecular Weight: 400.6 g/mol

9.2.4 Solubility: Insoluble in water, freely soluble in alcohol, chloroform, dioxane, ether, and soluble in vegetable oils

9.2.5 Description

Testosterone Enanthate Injection, for intramuscular injection, contains testosterone enanthate which is the oil-soluble ester of the androgenic hormone testosterone. Enanthate Injection is available as a colorless to pale yellow solution. Each mL contains 200 mg testosterone enanthate in sesame oil with 5 mg chlorobutanol as a preservative.

9.3 Clinical Pharmacology Testosterone Esters

Testosterone esters are less polar than free testosterone. Testosterone esters in oil injected intramuscularly are absorbed slowly from the lipid phase; thus, Testosterone Cypionate and Testosterone Enanthate can be given at intervals of two to four weeks.

Testosterone in plasma is 98 percent bound to a specific testosterone-estradiol binding globulin, and about 2 percent is free. Generally, the amount of this sex-hormone binding globulin in the plasma will determine the distribution of testosterone between free and bound forms, and the free testosterone concentration will determine its half-life.

About 90 percent of a dose of testosterone is excreted in the urine as glucuronic and sulfuric acid conjugates of testosterone and its metabolites; about 6 percent of a dose is excreted in the feces, mostly in the unconjugated form. Inactivation of testosterone occurs primarily in the liver. Testosterone is metabolized to various 17-keto steroids through two different pathways. The half-life of Testosterone Cypionate and Testosterone Enanthate when injected intramuscularly is approximately eight days. The two forms of the drug demonstrate identical pharmacokinetic properties.

9.4 Precautions

9.4.1 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.
3. Testosterone Cypionate or Enanthate should not be used interchangeably with testosterone propionate because of differences in duration of action.
4. Testosterone Cypionate and Testosterone Enanthate are not for intravenous use.

9.4.2 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.

9.4.3 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.

9.4.4 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.

9.4.5 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.

9.5 Adverse Reactions

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

1. Endocrine and urogenital: Gynecomastia and excessive frequency and duration of penile erections (priapism). Oligospermia may occur at high dosages.
2. Skin and appendages: Hirsutism, male pattern of baldness, seborrhea, and acne.
3. Fluid and electrolyte disturbances: Retention of sodium, chloride, water, potassium, calcium, and inorganic phosphates.
4. Gastrointestinal: Nausea, cholestatic jaundice, alterations in liver function tests, rarely hepatocellular neoplasms and peliosis hepatis.
5. Hematologic: Suppression of clotting factors II, V, VII, and X, bleeding in patients on concomitant anticoagulant therapy, polycythemia, thrombosis.

6. Nervous system: Increased or decreased libido, headache, anxiety, depression, and generalized paresthesia.
7. Allergic: Hypersensitivity, including skin manifestations and anaphylactoid reactions.
8. Miscellaneous: Inflammation and pain at the site of intramuscular injection.

9.5.1 Drug Abuse and Dependence

Controlled Substance Class:
Testosterone is a controlled substance under the Anabolic Steroids Control Act, and Testosterone Cypionate and Testosterone Enanthate Injection has been assigned to Schedule III.

9.5.2 Overdosage

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

9.6 Administration, Supply and Storage

9.6.1 Administration:

Testosterone Cypionate and Testosterone Enanthate injection is for intramuscular use only. It should not be given intravenously. Intramuscular injections should be given deep in the gluteal muscle.

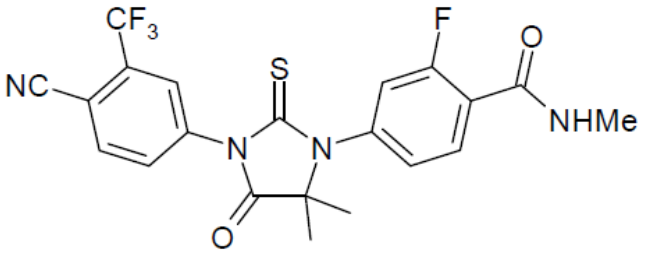
9.6.2 Supply

Testosterone Cypionate Injection, USP, 200 mg/mL is available as follows:
1 mL vials NDC 0574-0820-01
10 mL vials NDC 0574-0820-10
Testosterone Enanthate Injection, USP, 200 mg/mL is available as follows:
5 mL multi-dose vials NDC 67979-501-40
Testosterone will be used in the commercially available formulation, it will be purchased by the participant's site pharmacy; the cost will be covered by the study and will be provided to the patient at no charge.

9.6.3 Storage

Vials should be stored at controlled room temperature 20°C to 25°C (68°F to 77°F) [see USP]. Protect from light. Use carton to protect contents from light until used. Warming and rotating the vial between the palms of the hands will redissolve any crystals that may have formed during storage at low temperatures.

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.

9.7.1 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.

9.7.2 Safety/Precautions

9.7.2.1 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.

9.7.2.2 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.

9.7.2.3 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.

9.7.2.4 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.

9.7.2.5 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.

9.7.2.6 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.

9.7.2.7 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.
- 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.

9.7.2.8 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.

9.7.2.9 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.

9.7.2.10 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.

9.7.2.11 Administration, Supply and Storage

Enzalutamide is FDA approved for the indication under study.

9.7.2.12 Administration

Enzalutamide 160 mg daily (the current FDA approved dose), or four 40 mg capsules by mouth daily, will be administered. A 30 day supply will be provided at the beginning of each month of the trial.

9.7.2.13 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).
Enzalutamide will be used in the commercially available formulation as standard of care.

The cost for Enzalutamide will not be covered by the study.

9.7.2.14 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).

10 STUDY CALENDARS

10.1 ARM A: BAT with crossover to Enzalutamide

		Treatment Period (One Cycle is 28 days) (+/- 5 days)													
	Screening	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	After C10	End of Treatment	CROSSOVER	End of study visit[d,e]
	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11		Progression	CROSS-1	
History and Physical	X														
Clinic Visit	X	X	X	X	X	X	X	X	X	X	X	Q MONTH	X	Q 3C	X
ECOG PS	X	X	X	X	X	X	X	X	X	X	X	QMONTH	X	Q 3C	X
CBC	X		X		X			X			X	Q3C	X	Q 3C	X
COMP PANEL	X		X		X			X			X	Q3C	X	Q 3C	X
PSA	X		X	X	X	X	X	X	X	X	X	Q3C	X	Q MONTH	X
TESTOSTERONE	X		X		X			X			X	Q3C	X	Q 3C	
SHBG, DHT, Free Testosterone	X				X								X		
ESTRADIOL, DHEA-S, DHEA	X				X								X		
Fasting insulin,Fasting glucose	X				X			X			X	Q6C	X	Q 3C	
HGBA1c	X				X			X			X	Q6C	X	Q 3C	
C REACTIVE PROTEIN	X				X			X			X	Q6C	X	Q 3C	
C-TELOPEPTIDE	X				X			X			X	Q6C	X	Q 3C	
OSTEOCALCIN	X				X			X			X	Q6C	X	Q 3C	
LIPID PANEL	X				X			X			X	Q6C	X	Q 3C	
PT/PTT	X														
ECG	X														
BONE SCAN	X				X			X			X	Q3C		Q 3C	
CT SCAN[g]	X				X			X			X	Q3C		Q 3C	
^{18}F-DCFPyL PET/CT (JHU only)[a]	X				X										
AR CTC SAMPLE[b]	X				X								X[f]		
Tumor DNA AR Mutation[c]	X				X										
FACIT-F	X		X		X			X			X	Q3C	X	Q 3C	X
RANDSF-36	X		X		X			X			X	Q3C	X	Q 3C	X
IIEF SURVEY	X		X		X			X			X	Q3C	X	Q 3C	X
BRIEF PAIN INVENTORY	X		X		X			X			X	Q3C	X	Q 3C	X
PANAS SURVEY	X		X		X			X			X	Q3C	X	Q 3C	X
TESTOSTERONE		X	X	X	X	X	X	X	X	X	X	Q MONTH			
ENZALUTAMIDE														Q MONTH	

C= Cycle number; V=Visit number

[a] ^{18}F-DCFPyL PET/CT scans done at screening only in patients on BAT arm and then repeated after 3 cycles of BAT

[b] AR CTCs are collected screening, after 3 cycles of therapy on both arms and then at time of progression on Enza or BAT

[c] Sample collected for analysis of AR mutation in circulating tumor DNA at screening and after 3 cycles of therapy on BAT or Enzalutamide

[d] For those patients who choose to not cross-over and come off study treatment, they will come for an end of study visit within 30 days from their confirmed radiographic progression. For those patients who crossed over to receive the opposite arm of therapy, they will come for an end of study visit within 30 days from their disease progression (evidence of PSA progression per PCWG2 criteria or confirmed radiographic disease progression).

[e] For patients with clinical progression that requires patient to come off study (i.e. worsening pain, obstructive symptoms) end of study visit must be scheduled within 30 days of symptoms.

[f] At the time of progression, patients must also have blood drawn for CTCs to look for changes in AR-V7 and total AR expression. The CTCs samples will be either collected at the end of study visit for patients who opt not to cross-over, or at the cross over visit for patient who opt to cross over to receive the opposite treatments (CTCs samples will be collected before the start of the opposite therapy for cross over patients).

[g] CT scans should always include chest, abdomen and pelvis.

Note: Survival information needs to be collected. Refer to section 5.10

10.2 ARM B: Enzalutamide with crossover to BAT

		Treatment Period (One Cycle is 28 days) (+/- 5 days)													
	Screening	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	After C10	End of Treatment	CROSSOVER	End of study visit[c,d]
	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11		Progression	CROSS-1	
History and Physical	X														
Clinic Visit	X	X	X		X			X			X	Q3C	X	QMONTH	X
ECOG PS	X	X	X		X			X			X	Q3C	X	Q 3C	X
CBC	X		X		X			X			X	Q3C	X	Q 3C	X
COMP PANEL	X		X		X			X			X	Q3C	X	Q 3C	X
PSA	X		X	X	X	X	X	X	X	X	X	Q3C	X	Q MONTH	X
TESTOSTERONE	X		X		X			X			X	Q3C	X	Q 3C	
SHBG, DHT, Free Testosterone	X				X								X		
ESTRADIOL, DHEA-S, DHEA	X				X								X		
Fasting insulin,Fasting glucose	X				X			X			X	Q6C	X	Q 3C	
HGBA1c	X				X			X			X	Q6C	X	Q 3C	
C REACTIVE PROTEIN	X				X			X			X	Q6C	X	Q 3C	
C-TELOPEPTIDE	X				X			X			X	Q6C	X	Q 3C	
OSTEOCALCIN	X				X			X			X	Q6C	X	Q 3C	
LIPID PANEL	X				X			X			X	Q6C	X	Q 3C	
PT/PTT	X														
ECG	X														
BONE SCAN	X				X			X			X	Q3C		Q 3C	
CT SCAN[f]	X				X			X			X	Q3C		Q 3C	
AR CTC SAMPLE[a]	X				X								X[e]		
Tumor DNA AR mutation[b]	X				X										
FACIT-F	X		X		X			X			X	Q3C	X	Q 3C	X
RANDSF-36	X		X		X			X			X	Q3C	X	Q 3C	X
IIEF SURVEY	X		X		X			X			X	Q3C	X	Q 3C	X
BRIEF PAIN INVENTORY	X		X		X			X			X	Q3C	X	Q 3C	X
PANAS SURVEY	X		X		X			X			X	Q3C	X	Q 3C	X
TESTOSTERONE														Q MONTH	
ENZALUTAMIDE		X	X	X	X	X	X	X	X	X	X	Q MONTH			

C= Cycle number; V=Visit number

[a] AR CTCs are collected screening, after 3 cycles of therapy on both arms and then at time of progression on Enza or BAT

[b] Sample collected for analysis of AR mutation in circulating tumor DNA at screening and after 3 cycles of therapy on BAT or Enzalutamide

[c] For those patients who choose to not cross-over and come off study treatment, they will come for an end of study visit within 30 days from their confirmed radiographic progression. For those patients who crossed over to receive the opposite arm of therapy, they will come for an end of study visit within 30 days from their disease progression (evidence of PSA progression per PCWG2 criteria or confirmed radiographic disease progression).

[d] For patients with clinical progression that requires patient to come off study (i.e. worsening pain, obstructive symptoms) end of study visit must be scheduled within 30 days of symptoms.

[e] At the time of progression, patients must also have blood drawn for CTCs to look for changes in AR-V7 and total AR expression. The CTCs samples will be either collected at the end of study visit for patients who opt not to cross-over, or at the cross over visit for patient who opt to cross over to receive the opposite treatments (CTCs samples will be collected before the start of the opposite therapy for cross over patients).

[f] CT scans should always include chest, abdomen and pelvis.

Note: Survival information needs to be collected. Refer to section 5.10

11 DATA MONITORING AND REPORTING REQUIREMENTS

Data and safety monitoring will follow Level I under the SKCCC Data and Safety Monitoring Plan (DSMP, 12/6/2012). Data Monitoring of this protocol will occur on a regular basis with the frequency dependent on the rate of subject accrual and the progress of the study. The protocol will be monitored internally at SKCCC by the Principal Investigator and externally by the SKCCC CRO in accordance with SKCCC guidelines. Trial monitoring and reporting will be done through the Safety Monitoring Committee (SMC) at SKCCC.

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: 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.

11.1 Case Report Form Submission

Data required by the study will be collected in Case Report Forms provided by the Coordinating Center at SKCCC. The participating site will be required to complete a paper Eligibility Checklist case report form (CRF) at the time of patient registration (please refer to section 5.1 Initial Registration Process). All other data will be collected on the electronic case report forms (eCRFs) in CRAB (Cancer Research And Biostatistics) system. Site staff access to CRAB will be initiated at the time of the site activation.

Case Report Form Completion

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 via to the TRANSFORMER Study Manager, Haiyi (Harry) Cao, at hcao7@jhmi.edu. 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.

Authorized representatives of the Coordinating Center (SKCCC) may visit the satellite sites to perform audits or inspections, including source data verification. The purpose of these audits or inspections is to systematically and independently examine all trial-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice (GCP), and any applicable regulatory requirements.

11.2 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, 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 cypionate, testosterone enanthat 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).

11.3 Evaluating Adverse Events

The grade and severity of the event will be determined using the DCT/NCI Common Terminology Criteria, CTCAE v.4.0. Links to CTCAE version 4.0 can be found in Appendix 2. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. Study staff must use one of the CTCAE criteria to define the event. Adverse events not included in the CTCAE v.4.0 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).
- *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.

11.3.1 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.

11.4 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.

11.5 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: http://www.hopkinsmedicine.org/institutional_review_board/guidelines_policies/organization_policies/103_6b.html

All deaths on study regardless of attribution must be reported by the Principal Investigator Dr. Denmeade or Co-Principal Investigator Dr. Eisenberger to the JHM IRB. In addition, all serious adverse events, regardless of causality to study drug, will be reported promptly to the TRANSFORMER Study Manager, Haiyi (Harry) Cao, at hcao7@jhmi.edu 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/Co-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.

11.6 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.

11.7 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.

11.8 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.

The following must appear in consent form for each participating site:

- A statement that the DOD or a DOD organization is funding the study
- A statement that representatives of the DOD are authorize to review research records
- In the HIPAA Authorization section of the consent form, representatives of the DOD must be listed as one of the parties to whom private information may be disclosed
- Because the study involves a research monitor, the monitor should also be listed in the HIPAA Authorization.

11.9 Multicenter Guidelines

The Protocol Chair

The Protocol Chair, Samuel Denmeade, MD, is 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 current IRB approved 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) from all sites
- Reviewing all study data from all sites

Coordinating Center Responsibilities (SKCCC)

Coordinating Centers must:

- Verify that each participating institution has a Federal Wide Assurance (FWA) number.
- Confirm that IRB approval has been obtained at each participating site prior to their first patient registration
- Maintain copies of IRB approvals from each site
- Implement central patient registration
- Prepare all submitted data for review by the Protocol Chair (Samuel Denmeade, MD)
- Establish procedures for documentation, reporting, and submitting of adverse events to the Protocol Chair (Samuel Denmeade, MD) and all applicable parties
- Facilitate audits by securing selected source documents and research records from participating sites for audit, or by conducting audits at participating sites.

Participating Sites

Participating sites are responsible for performing the following tasks:

- Follow the protocol as written and conduct the study within the guidelines of Good Clinical Practice.
- Collect and submit data, and report adverse events according to the schedule specified by the protocol.
- Register all patients with the Lead Center (SKCCC) by submitting patient registration forms, and signed informed consents promptly.
- Provide sufficient experienced clinical and administrative staff; as well as adequate facilities and equipment to conduct a collaborative trial according to the protocol.
- Maintain regulatory binders on site, and provide copies of all required documents to the Lead Center (SKCCC)

12 STATISTICAL METHODS

This is a multi-center, open label, randomized study to evaluate the efficacy of bipolar androgen therapy (BAT) vs. enzalutamide in asymptomatic men with castration resistant metastatic prostate cancer post-treatment with abiraterone. Eligible patients will be randomized in a 1:1 ratio to one of two treatment arms: BAT (Arm A) or enzalutamide (Arm B). The randomization will be stratified by duration of abiraterone acetate therapy (<6 months *vs* ≥6 months).

Primary objective and primary endpoint: The primary objective of the study is to compare progression-free survival (PFS) between BAT and enzalutamide in patients with castration resistant prostate cancer who have progressed on abiraterone. The primary efficacy endpoint is PFS, defined as the time from the date of the randomization to the date of first documented clinical progression due to prostate cancer or radiological confirmation of progression per RECIST 1.1 for soft tissue or PCWG2 for bone lesions or death, whichever occurs first. If a patient has not had the event at the date of the analysis cut-off, PFS will be censored at the time of the last adequate tumor assessment before the cut-off date.

Sample size and power considerations: No studies have been published that describe PFS on enzalutamide after progression on abiraterone in chemo-naive patients. In the trial of abiraterone plus prednisone in men with metastatic prostate cancer without previous chemotherapy the rPFS was 16.5 months vs 8.3 months for the prednisone alone arm. In the phase III trial of enzalutamide in men with metastatic prostate cancer after docetaxel chemotherapy, the rPFS was 8.3 months. In the study of Shrader et al (n=35) and in our own unpublished analysis (n=41), the rPFS for patients receiving enzalutamide after Abiraterone (pre- or post-chemotherapy) was 4.9 and 4.2 months respectively. Based on these collective results, we have made the reasonable assumption that the median rPFS in the enzalutamide arm post Abiraterone will be 6 months. This trial is designed to detect a 50% improvement in median PFS in the BAT group, from 6 to 9 months (corresponding to a HR 0.667 of BAT vs. enzalutamide), at one-sided significance level of 0.05. Allowing for the interim analysis plan discussed below, the study requires at least 156 events to ensure a sequential test procedure will have 80% power. We expect a recruitment period of 24 months and an additional 12 months of follow-up. After accounting for an estimated 15% loss to follow-up, a total of up to 194 patients (97 per arm) will be randomized to record 156 events in 36 months of study. Recruitment for the study will stop once a total of 156 events have occurred. The calculation was performed using EAST® software.

Interim analysis: Two interim analyses for PFS are planned that allow the study to stop for efficacy. The first interim analysis will be conducted after approximately 45% of the information (i.e. 70 events of progression) has been recorded, and the second interim analysis is planned after 70% of the information (109 events) has been observed. An α-spending function method of Lan and DeMets with an O'Brien-Fleming-type stopping boundary, will be used to construct the stopping boundaries for the efficacy analyses.

The details of the planned interim analysis can be found in the table below.

Interim and Final Analysis	Percent Information	Estimated Upper Boundary for Declaring Superiority	Hazard Ratio cut-off	Approximate Time (Months)	Estimated Number of Events
1	45	-2.7031	0.525	18	70
2	70	-2.098	0.670	24	109
3 (Final)	100	-1.705	0.761	36	156

The study will also be monitoring for early stopping for futility using Jennison-Turnbull repeated confidence interval methodology (56). At each interim analysis the nominal (1- 2 × alpha) confidence interval on the rPFS hazard ratio comparing the BAT arm to the enzalutamide arm (arm A versus B) will be computed, where alpha is the nominal one-sided significance level of the boundary from the error spending function at the information fraction for the particular analysis time. If the confidence interval does not contain the target alternative HR of 0.667, then the data monitoring committee may consider terminating the study early for lack of treatment differences. The monitoring plan has negligible impact on the power of the study.

Toxicity monitoring: We will be monitoring adverse event of pain on the BAT arm, after every cohort of 20 patients has been treated. If the toxicity of grade 3 or higher pain appears to be higher than 20%, we will temporarily halt the study pending safety evaluations. Specifically, we will apply a Bayesian toxicity monitoring rule that suspends the enrollment if the posterior probability of risk being larger than that threshold is 75% or higher. Based on the data of previous studies of T, monitoring rule uses Beta (1, 10) as prior distribution. This means that our prior estimate of proportion of severe pain is 9%, and there is 90% chance that this proportion is 0.5%-26%. The decision rule for safety stopping is as follows:

Stop if:

# severe pain >=	6	10	13	16	19	23
Out of	15	30	45	60	75	90

The operating characteristic of the stopping rule based on 10,000 simulations:

True rate of pain	0.1	0.15	0.2	0.25	0.3	0.35
% simulated trials declaring unsafe	0.2	3.9	25.1	63.4	90.7	98.8

Analysis of primary endpoint: The comparison of PFS will be an intent-to-treat analysis including all randomized patients. The PFS will be estimated using Kaplan-Meier method, and the median PFS along with 95% confidence intervals (CI) will be reported by the treatment groups. The PFS will be compared between the two arms using a stratified log-rank test with the stratification factor of duration of prior abiraterone treatment. The Cox regression model, stratified for the same baseline stratification factor, will be used to estimate the hazard ratio (HR) of PFS between the two arms and corresponding 95% CI. Additionally, a Cox regression model, stratified for the baseline stratification factor, will be used to explore the potential influences of the other factors on the primary endpoint PFS.

Analysis of secondary efficacy endpoints: Time-to-event endpoints including progression-free survival based on radiographic or clinical progression, time to initiation of chemotherapy (i.e. time from the date of randomization to the date of initiation of docetaxel chemotherapy), and time to PSA progression will be analyzed similarly as described for the primary endpoint of PFS. Overall response, defined as the proportion

of subjects who achieve either complete response or partial response per RECIST 1.1, will be tabulated for each arm and compared using Chi-square test. Percent PSA change from baseline for individual patients will be presented in waterfall plots. PSA response rate will be estimated as the proportion of subjects who have any decrease in PSA below baseline and as the percentage with ≥50% PSA reduction from baseline level, and compared between the two arms using Chi-square test. Percent PSA change from baseline for individual patients will be presented in waterfall plots and the changes over time will be shown using spaghetti plots. On the BAT arm among the patients who repeat ADT post-BAT treatment, we will assess the absolute and percent change of PSA levels from the last PSA on BAT to nadir PSA on return to castrate T levels. To examine the effect of BAT to sensitize patients for enzalutamide, we will estimate the objective response rate and PSA response among those who subsequently receive enzalutamide on the BAT arm. PFS2 is defined as time from randomization to progression on crossover treatment. For those who do not receive crossover treatment, PFS2 is censored at the end of the initial treatment. PFS2 will be summarized using Kaplain-Meier method in each arm, and compared between the two arms in intent-to-treat population using log-rank test. PFS2 will also be analyzed among those who cross over. Overall survival, defined as time from randomization to death for any cause, will be characterized using Kaplan-Meier method and compared between the two arms using log-rank test.

Analysis of quality of life and metabolic studies: QoL will be assessed using RAND-SF36 Quality of Life Survey, FACIT-F Version 4, I-PANAS-SF. Sexual capacity, functional capacity and pain will be assessed using IIEF, Global Assessment of Change and BPI, respectively. For each module, summary statistics of the scores will be reported at baseline randomization, one month post-randomization, three months post-randomization, six months, and 12 months post-randomization. In each arm 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. bone density, lipid profile, metabolic panel, steroid panel, inflammation/cytokines, BMI, lean mass) will be evaluated using the same analysis methods. We will also compare the metabolic parameters between the two arms using regression models.

Analysis populations:
The Intent-to-Treat (ITT) Population includes all subjects who are randomized with study treatment assignment designated according to initial randomization. The ITT population will be the primary population for evaluating efficacy endpoints (e.g. PFS, time to PSA progression, ORR, PSA response) and patient characteristics.

Per Protocol Population will include all patients who are randomized and received at least one dose of study medication, and do not have any major protocol violations (for example, incorrect treatment group allocation according to randomization, not meeting the major inclusion/exclusion criteria for disease status and prior therapies, noncompliance to treatment plan or assessment schedule; additional concurrent prohibited therapies). The per-protocol analysis will serve as supportive.

Safety Population will include all patients who receive at least one dose of study medication, with treatment assignments designated according to actual study treatment received. The safety analyses population will be the primary population for evaluating treatment administration/compliance and safety.

Safety analysis: 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 4.0) grade.

AR-variant analysis: We will determine the association between BAT-induced decrease in AR-V7 expression and restoration of sensitivity to ADT and enzalutamide. We will examine the association of the AR-V7 positivity at baseline with clinical outcomes, including PFS, objective response rate (ORR) and PSA response, from patients receiving BAT or enzalutamide. For the time-to-event outcome PFS, Kaplan-Meier curves will be plotted for each treatment arm stratified by baseline AR-V7 status. Cox proportional hazards models will be used to estimate the hazard ratio with 95% Confidence Intervals for each arm to quantify the effect of AR-V7 presence/absence on the risk of failures. The binary outcomes ORR and PSA response will be compared between baseline AR-V7(+) and AR-V7(–) groups using chi-square tests. We will also explore the differential effect of BAT vs. enzalutamide in AR-V7 (+) and AR-V7(-) group by examining the interaction of AR-V7 and treatment group in the Cox model for PFS.

Power considerations: Statistical power is based on the primary analysis of the association of AR-V7 and PFS. Based on our previous work, we expect AR-V7 to be present at baseline in approximately 30% of patients with CRPC. We anticipate having baseline blood samples from 160 patients (80 per arm) for AR-V7 analysis. Our hypothesis is that AR-V7 drives aberrant AR activity after the canonical AR axis is blocked by the AR-targeted therapies; therefore patients who are AR-V7(+) at baseline in the enzalutamide arm will be expected to have a higher risk of disease progression. For the risk difference to be clinically meaningful, we are interested in detecting a hazard ratio of 2.5 among AR-V7(+) patients compared to AR-V7(–) patients. Assuming median PFS to be about 6 months in the enzalutamide arm, a sample size of 80 patients with blood samples evaluable for molecular CTC analyses (24 AR-V7(+) and 56 AR-V7(-)) would achieve 90% power to detect a hazard ratio of 2.5 based on a 2-sided log-rank test at a significance level of 0.05. This corresponds to a median PFS of 4.2 mos and 10.5 mos for patients receiving enzalutamide with AR-V7(+) and AR-V7(–) tumors, respectively.

AR mutation analysis: We will examine AR mutations in ctDNA and evaluate the change of AR mutation post-BAT treatment compared to baseline.

Power considerations: We hypothesize that the mutations of interest will disappear after BAT therapy. We hope to determine if more than 10% patients who carry certain mutations at baseline would lose the mutation after the treatment of BAT, and expect that at least 50% of them would turn to mutation negative. A sample size of 14 patients with positive mutations at baseline provides 91% power to reject 10% rate of conversion from positive to negative in favor of 50% conversion rate, using a one-sided binomial test at significance level 0.025. Assuming the mutation rate is around 25% at baseline, we will test 60 patients before BAT therapy in order to have at least 14 positives at baseline for us to evaluate the change of their mutation status with the treatment of BAT.

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. Shah RB, et al. Androgen-independent prostate cancer is a heterogeneous group of diseases: lessons from a rapid autopsy program. Cancer Res 2004; 64: 9209-16.
8. 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.
9. 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.
10. 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.
11. 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
12. 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.
13. Ryan CJ, Smith MR, de Bono JS, Molina A, Logothetis CJ, de Souza P, Fizazi K, Mainwaring P, Piulats JM, Ng S, Carles J, Mulders PF, Basch E, Small EJ, Saad F, Schrijvers D, Van Poppel H, Mukherjee SD, Suttmann H, Gerritsen WR, Flaig TW, George DJ, Yu EY, Efstathiou E, Pantuck A, Winquist E, Higano CS, Taplin ME, Park Y, Kheoh T, Griffin T, Scher HI, Rathkopf DE; COU-AA-302 Investigators. Abiraterone in metastatic prostate cancer without previous chemotherapy. N Engl J Med. 2013;368:138-48.

14. Beer TM, Armstrong AJ, Rathkopf DE, Loriot Y, Sternberg CN, Higano CS, Iversen P, Bhattacharya S, Carles J, Chowdhury S, Davis ID, de Bono JS, Evans CP, Fizazi K, Joshua AM, Kim CS, Kimura G, Mainwaring P, Mansbach H, Miller K, Noonberg SB, Perabo F, Phung D, Saad F, Scher HI, Taplin ME, Venner PM, Tombal B; the PREVAIL Investigators. Enzalutamide in Metastatic Prostate Cancer before Chemotherapy. N Engl J Med. 2014 Jun 1. [Epub ahead of print]
15. 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 USA 1996;93:11802-07. PMCID:PMC38139
16. Litvinov IV, Vander Griend DJ, Antony L, Dalrymple S, De Marzo AM, Drake CC, Isaacs JT. Androgen receptor as a licensing factor for DNA replication in androgen-sensitive prostate cancer cells. Proc Natl Acad Sci 2006;103:15085-90. PMCID:PMC1622781
17. Vander Griend DJ, Litvinov IV, Isaacs JT. Stabilizing androgen receptor in mitosis inhibits prostate cancer proliferation. Cell Cycle 2007;6:647-51.
18. 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
19. 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
20. 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.
21. 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
22. 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.
23. 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.
24. 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.
25. 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]
26. 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]

27. Zhang T, Dhawan, S, Healy P, George DJ, Oldan J, Chin B, Armstrong A. Clinical benefit of docetaxel or enzalutamide after progression on first-line abiraterone acetate and prednisone in men with metastatic castration resistant prostate cancer (mCRPC). J Clin Onc 2014;32:suppl abst e16031.
28. Suzman D, Schweizer M, Luber B, Nadal R, Antonarakis E. Clinical activity of enzalutamide versus docetaxel in men with castration resistant prostate cancer progressing after abiraterone. Prostate, In Press, 2014.
29. Hu R, Denmeade SR, Luo J. Molecular processes leading to aberrant androgen receptor signaling and castration resistance in prostate cancer. Expert Rev Endocrinol Metab. 2010;5:753-764. PMCID:PMC3035007
30. Antonarakis E, et al. N Engl J Med. 2014; In Press.
31. Brendler H, Chase WE, Scott WW. Prostatic Cancer: Further investigation of hormone relationships. Arch Surg 1949;433-440.
32. Prout GR, Brewer WR. Response of men with advanced prostatic carcinoma to exogenous administration of testosterone. Cancer 1967;20:1871-1878.
33. Donati RM, Ellis H, Gallagher NI. Testosterone potentiated 32P therapy in prostatic carcinoma. Cancer 1966;19:1088-90.
34. Johnson DE, Haynie TP. Phosphorus-32 for intractable pain in carcinoma of prostate: Analysis of androgen priming, parathormone rebound, and combination therapy. Urology 1977;9:137-39.
35. Manni A, Bartholomew M, Caplan R, Boucher A, Santen R, et al. Androgen Priming and Chemotherapy in Advanced Prostate Cancer: Evaluation of Determinants of Clinical Outcome. J Clin Oncol 1988;6:1456-1466
36. 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]
37. 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.
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. 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.
40. Bergerat J, Ceraline J. Pleiotropic functional properties of androgen receptor mutants in prostate cancer. Hum Mutat. 2009;30:145-57.
41. Beltran H, Yelensky R, Frampton GM, Park K, Downing SR, MacDonald TY, Jarosz M, Lipson D, Tagawa ST, Nanus DM, Stephens PJ, Mosquera JM, Cronin MT, Rubin MA. Targeted next-generation sequencing of advanced prostate cancer identifies potential therapeutic targets and disease heterogeneity. Eur Urol. 2013;63:920-6. PMCID:PMC3615043
42. Jiang Y, Palma JF, Agus DB, Wang Y, Gross ME. Detection of androgen receptor mutations in circulating tumor cells in castration-resistant prostate cancer. Clin Chem. 2010;56:1492-5.
43. Veldscholte J, Ris-Stalpers C, Kuiper GG, Jenster G, Berrevoets C, Claassen E, et al. 1990. A mutation in the ligand binding domain of the androgen receptor of human LNCaP cells affects steroid binding characteristics and response to anti-androgens. Biochem Biophys Res Commun 1990;173:534.

44. Haapala K, Hyytinen ER, Roiha M, Laurila M, Rantala I, Helin HJ, et al. Androgen receptor alterations in prostate cancer relapsed during a combined androgen blockade by orchiectomy and bicalutamide. Lab Invest 2001;81:1647.
45. Hara T, Miyazaki J, Araki H, Yamaoka M, Kanzaki N, Kusaka M, et al. Novel mutations of androgen receptor: a possible mechanism of bicalutamide withdrawal syndrome. Cancer Res 2003;63:149.
46. Balbas MD, Evans MJ, Hosfield DJ, Wongvipat J, Arora VK, Watson PA, Chen Y, Greene GL, Shen Y, Sawyers CL. Overcoming mutation-based resistance to antiandrogens with rational drug design. Elife. 2013 Apr 9;2:e00499. PMCID:PMC3622181
47. Korpal M, Korn JM, Gao X, Rakiec DP, Ruddy DA, Doshi S, Yuan J, Kovats SG, Kim S, Cooke VG, Monahan JE, Stegmeier F, Roberts TM, Sellers WR, Zhou W, Zhu P. An F876L mutation in androgen receptor confers genetic and phenotypic resistance to MDV3100 (Enzalutamide). Cancer Discov. 2013;3:1030-43.
48. Joseph JD, Lu N, Qian J, Sensintaffar J, Shao G, Brigham D, Moon M, Maneval EC, Chen I, Darimont B, Hager JH. A Clinically Relevant Androgen Receptor Mutation Confers Resistance to Second-Generation Antiandrogens Enzalutamide and ARN-509. Cancer Discov. 2013;3:1020-9.
49. Dressman D, Yan H, Traverso G, Kinzler KW, Vogelstein B. Transforming single DNA molecules into fluorescent magnetic particles for detection and enumeration of genetic variations. Proc Natl Acad Sci USA 2003;100:8817-22. PMCID:PMC166396
50. Kinde I, Papadopoulos N, Kinzler KW, Vogelstein B. FAST-SeqS: a simple and efficient method for the detection of aneuploidy by massively parallel sequencing. PLoS One. 2012;7:e41162.
51. Ghosh A, Heston WD. Tumor target prostate specific membrane antigen (PSMA) and its regulation in prostate cancer. J Cell Biochem. 2004;91:528-39.
52. Noss KR, Wolfe SA, Grimes SR. Upregulation of prostate specific membrane antigen/folate hydrolase transcription by an enhancer. Gene. 2002 Feb 20;285(1-2):247-56.
53. Cho, S. Y., Gage, K. L., Mease, R. C., Senthamizhchelvan, S., Holt, D. P., Jeffrey-Kwanisai, A., Endres, C. J., Dannals, R. F., Sgouros, G., Lodge, M., Eisenberger, M. A., Rodriguez, R., Carducci, M. A., Rojas, C., Slusher, B. S., Kozikowski, A. P., and Pomper, M. G. Biodistribution, tumor detection, and radiation dosimetry of 18F-^{18}F-DCFPyL, a low-molecular-weight inhibitor of prostate-specific membrane antigen, in patients with metastatic prostate cancer. J Nucl Med 2012;53;1883-1891.
54. Jennison C, Turnbull BW. Sequential equivalence testing and repeated confidence intervals, with applications to normal and binary responses. Biometrics. 1993;49:31-43.
55. Chen Y, Pullambhatla M, Byun Y, et al. 2-(3-{1-Carboxy-5-[(6-[18F]fluoro-pyridine-3-carbonyl)-amino]-pentyl}-urei do)-pentanedioic acid, [18F]DCFPyL, a PSMA-based PET Imaging Agent for Prostate Cancer. *Clin Cancer Res.* Oct 31 2011.

Appendix I

Response Evaluation Criteria in Solid Tumors Version 1.1
Definitions
Baseline: Baseline is defined as the most recent assessment performed prior to randomization.
Baseline assessments must be performed within the period defined in the protocol eligibility criteria.

Measurable lesions: Except for lymph nodes as described below, measurable lesions are defined as those that can be accurately measured in at least 1 dimension (longest diameter to be recorded) as ≥ 10 mm with CT scan (if CT scans have slice thickness greater than 5 mm the minimum size for a measurable lesion is twice the slice thickness).

- To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and recorded.
- MRI may be substituted for contrast-enhanced CT for lesions at some anatomical sites, but not for lesions in the lungs. The minimum size for measurability is the same as for CT (10mm) as long as the scans are performed with slice thickness of 5 mm and no gap. If MRI is performed with thicker slices, the size of a measurable lesion at baseline should be twice the slice thickness. In the event there are interslice gaps, this also needs to be considered in determining the size of measurable lesions at baseline.

Nonmeasurable lesions: All other lesions (or sites of disease), including small lesions (longest diameter < 10 mm or pathological lymph nodes with ≥10 to < 15 mm short axis), are considered nonmeasurable. Lymph nodes that have a short axis < 10 mm are considered nonpathological and are not be recorded or followed. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/ pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as nonmeasurable.

Target lesions: All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, are to be identified as **target lesions** and measured and recorded at baseline. Target lesions are to be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, and be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. Target lesions will be measured at each assessment (longest axis for nonnodal lesions, shortest axis for measurable malignant nodal lesions).

Nontarget lesions: All other lesions (or sites of disease) including all non-measurable lesions (including pathological lymph nodes with ≥ 10 to <15 mm short axis) and all measurable lesions over and above the 5 target lesions are to be identified as **non-target lesions** and recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each is to be recorded throughout follow-up. Lymph nodes thathave a short axis < 10mm are considered non-pathological and are not to be recorded or followed.

Methods of Measurement

The same method of assessment and the same technique used to characterize each identified and reported lesions at baseline should be used during each follow-up assessment. All measurements should be taken and recorded in metric notation using a ruler or calipers. Imaging based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but assessed by clinical examination (referring to biopsy-proven visible lesion(s) on the chest).

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.

Time Point Assessments

The frequency and schedule of tumor assessments is defined in the protocol. The schedule is to be maintained regardless of whether study treatment is held or discontinued.
At baseline, tumors and lymph nodes are classified and documented as target or nontarget per the definitions provided above. It is possible to record multiple nontarget lesions involving the same organ as a single item (eg, 'multiple liver metastases'). At all postbaseline (follow-up) evaluations the baseline classification (target, nontarget) is to be maintained and lesions are to be documented and described in a consistent fashion over time (eg, recorded in the same order on source documents).

At each assessment, a sum of the diameters (longest for nonnodal lesions, short axis for nodal lesions) for all target lesions will be calculated and included in source documents. The *baseline sum of the diameters* (SoD) will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease. The lowest SoD (nadir) since (and including) the baseline value will be used as reference for evaluating progression.

After baseline, target lesions should have the actual size documented, if possible, even if the lesions become very small. If in the opinion of the radiologist the lesion has likely disappeared, 0 mm should be recorded. If the lesion is present but too small to measure, an indicator for 'too small to measure' this should be included in source documents.

For target lesions, measurements should be taken and recorded in metric notation. All tumor measurements must be recorded in millimeters.

Nontarget lesions are to be assessed qualitatively (present, resolved, or unequivocal progression) and new lesions, if any, are to be documented separately.

At each evaluation, progression status is to be determined based upon the time point status for target lesions, nontarget lesions, and new lesions.

Response Criteria

Evaluation of Target Lesions

Complete Response (CR):	Disappearance of all target lesions. All pathological lymph nodes(whether target or non-target) must have reduction in short axis to < 10 mm.
Partial Response (PR):	At least a 30% decrease in SoD of target lesions, taking as a reference the baseline SoD
Progressive Disease (PD):	At least a 20% increase in the SoD of target lesions, taking as a reference the smallest (nadir) SoD since (and including) baseline. In addition to the relative increase of 20%, the SoD must also demonstrate an absolute increase of at least 5 mm.
Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD
Not Applicable (NA)	No target lesion identified at baseline.
Unable to Evaluate (UE)	One or more target lesions are not imaged and the remainder of the SoD compared with the nadir SoD does not meet the criterion for PD.

Evaluation of Non-Target Lesions

Complete Response (CR):	Disappearance of all non-target lesions. All lymph nodes must be nonpathological in size (<10 mm short axis)
Non-CR / Non-PD:	Persistence of one or more non-target lesion(s).
Progressive Disease (PD):	Unequivocal progression of non-target lesions. Unequivocal progression should normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase
Not Applicable (NA)	No non-target lesions identified at screening
Unable to Evaluate (UE)	One or more non-target lesions are not imaged and the remaining nontarget lesions do not meet the criterion for PD.

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).

New Lesion Time Point Response (TPR)

Yes	Lesion present at follow-up visit either for the very first time or reappearing (ie, lesion was present at baseline, disappeared at a follow-up visit and re-appeared later). On bone scan, a single new lesion may not be sufficient to qualify as PD. Confirmation should be obtained by performing CT or MRI of the area of concern to confirm ambiguous results of bone scan. Preferred method for confirmation is MRI.
No	No new lesions present at follow-up.
Unable to Evaluate (UE)	Subject not assessed or incompletely assessed for new lesions.

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 Lesion TPR	Non-target lesion TPR	New lesion TPR	Overall TPR
CR	CR or NA	No	CR
CR	Non-CR/non-PD	No	PR
CR	UE	No	PR
PR	Non-PD or NA or UE	No	PR
SD	Non-PD or NA or UE	No	SD
UE	Non-PD	No	UE
PD	Any	No or Yes or UE	PD
Any	PD	No or Yes or UE	PD
Any	Any	Yes	PD
NA	CR	No	CR
NA	Non-CR/non-PD	No	Non-CR/non-PD
NA	UE	No	UE
Non-PD	Non-PD	UE	UE

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 2

NCI COMMON TOXICITY CRITERIA, VERSION 4.0

Version 4.0 of the NCI CTC, dated May 28, 2009, may be viewed and/or downloaded by accessing the following websites
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcae_4_with_lay_terms.pdf

http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

Appendix 3

NCCN Guidelines for Adult Cancer Pain (Version 1.2014) can be may be viewed and/or downloaded by accessing the following website:

http://oralcancerfoundation.org/treatment/pdf/pain.pdf

Appendix 4

Blood Collection and Shipping for TRANSFORMER Study

Blood draws should be scheduled on Monday-Thursday. Samples will be shipped the same day of blood collection to allow overnight delivery on weekdays before the weekend.

Two sets of blood samples for AR and AR-V7 will be collected at screening visit, cycle 4 (visit 5) visit, and at the visit where patients have radiographic progression on CT or bone scan:

Instructions for First Set of Blood Samples (AR):

1. Collect Blood in two Becton Dickinson (BD) Vacutainer ACD Solution A tubes (BD ACD-A). Product number for the BD ACD-A tube is 364606.

2. Ensure that at least 8.5 mL of blood is drawn into each tube. Avoid low volume to minimize agitation during shipping.

3. Invert the tubes gently 180 degree and back 3-4 times.

4. Store samples until shipping at 4-8^{o}C in a refrigerator.

Instructions for Second Set of Blood Samples (AR-V7):

1. Collect 2.5 mL blood into each of the two 5 mL PAXgene tubes. Product number from Becton Dickinson is 762165.

2. Invert the tubes gently 180 degrees and back 8-10 times.

3. Maintain blood at room temperature (6^{o}C to 37^{o}C) until shipping. Do Not place tubes in Refrigerator or Freezer.

One set of blood samples for Circulating Tumor DNA will be collected at screening visit, cycle 4 (visit 5) visit:

Instructions for Circulating Tumor DNA Blood Samples:

1. Collect blood in two Streck Cell-Free DNA BCT tubes. Product number from Streck, Inc., Omaha NE is 218961.

2. Ensure that at least 10 mL of blood is drawn into each tube. Avoid low volume to minimize agitation during shipping.

3. Invert the tubes gently 180 degree and back 8-10 times.

4. Maintain Blood at room temperature (6°C to 37°C) until shipping. Do Not place tubes in Refrigerator or Freezer.

Instructions for Shipping:

1. Activate the small standard duration FEDEX cold box (purchased from http://orderboxesnow.com) and place the tubes wrapped in bubble wraps snugly in the shipping box.

2. Complete the shipping manifest and ship the six tubes (2 tubes Circulating Cells, 2 tubes Circulating Tumor DNA, 2 tubes whole blood PAXgene) by FEDEX to the address below the same day of blood draw. Notify the recipient of the tracking number on the day of shipment by email.

The Fedex cold boxes are available at http://orderboxesnow.com. Bubble wraps can be purchased from Staples or other shipping supply stores.

Jun Luo, Ph.D.
415A Marburg, Johns Hopkins Hospital
600 N. Wolfe Street
Baltimore, MD 21287
Tel: 443-287-5625

Email shipping notifications to:
jluo1@jhmi.edu
denmesa@jhmi.edu
hcao7@jhmi.edu

Appendix 5

^{18}F-DCFPyL PET/CT Imaging (JHU only)

1. Patients must be enrolled on either arm of the randomized study and receiving treatment at Johns Hopkins.
2. A total of up to 20 patients will be enrolled. Once twenty patients are enrolled recruitment of patients will stop.
3. Patients will undergo ^{18}F-DCFPyL-PET prior to starting BAT or enzalutamide and after 12 weeks of treatment on either arm.
4. Patients must have Radiologic evidence of new or progressive metastatic prostate cancer demonstrated on CT imaging or bone scintigraphy.
5. Patient must be able to tolerate lying flat for the duration of the PET/CT scan (up to 60 minutes)
6. Patient should not eat or drink (except for water) for at least six hours prior to DCFpyL administration. Drinking water for hydration is encouraged. Multi- vitamins and folic acid supplements should also not be take the day of ^{18}F-DCFPyL PET/CT imaging.
7. An intravenous peripheral intravenous catheter is placed (or an existing central line accessed in the arms or hands only; no lower extremity peripheral lines will be used) to inject the radioactive tracer.
8. Vital signs (blood pressure, heart rate, respiratory rate, pulse oximetry), height, weight will be taken prior to administration of the radiotracer.
9. Intravenous fluid 5% dextrose + 0.45% normal saline at 15 ml/kg (maximum 1000 ml) delivered over 30 to 60 minutes prior to the injection of the radiopharmaceutical. The intravenous infusion is continued at a low flow rate for the remainder of the study.
10. PET/CT will be performed on a Discovery DRX PET/CT scanner (GE Healthcare) operating in 3-dimensional emission PET acquisition mode and using CT for attenuation correction. There will be an initial CT scan for attenuation correction followed by two successive PET scans, attenuation corrected to the same initial CT.
11. A bolus of less than or equal to 9 mCi (333 MBq) of ^{18}F-DCPyL will be injected into the IV line by slow IV push.
12. At approximately 60 minutes after the administration of ^{18}F-DCFPyL, a whole-body CT and PET scan will be acquired from the vertex of skull to mid-thigh. PET/CT will be performed on a Discovery DRX PET/CT scanner (GE Healthcare) operating in 3-dimensional emission acquisition mode. Scans will include approximately 8 to 9 fields-of-view, depending on patient height. Images will be reconstructed as per routine clinical PET/CT scan by OSEM reconstruction algorithm.
13. Vital signs (blood pressure, heart rate, pulse oximetry) will be taken within 1 hour after completion of PET/CT imaging.
14. Peripheral IV catheter will be removed by radiology staff prior to patient leaving the PET center.

General Concomitant Medication and Supportive Care Guidelines

There are no known contraindicated medications.

Post-Imaging Evaluation

The research nurse will contact the patient around 7 days (3 - 10 days) after the PET/CT study to inquire about delayed side effects. If there are suspected problems the patient will be asked to return for a clinic visit for a follow-up clinic visit

Image Acquisition, Archiving, and Interpretation

1. PET/CT will be performed on a Discovery DRX PET/CT scanner (GE Healthcare) operating in 3-dimensional emission acquisition mode and using CT for attenuation correction. Patients will be scanned in the supine position of the pelvis or starting from the vertex of the skull to mid-thigh (whole body protocol) which will include approximately 8 to 9 fields-of-view, depending on patient height. An initial low dose x-ray CT transmission scan, preceding the serial PET emission acquisitions, will be used for tissue attenuation correction and anatomical correlation. PET/CT images will be stored on our institutional radiology PACS system. PET/CT images will be anonymized for image analysis on a rolling basis. Data from a series of the dynamic pelvic PET scan and whole body PET will be analyzed. PET images will be analyzed on the GE Advanced Workstation software. Reconstruction of the PET data will be performed by means of iterative reconstruction (IR) by the ordered subset-expectation- maximization (OSEM) method with CT attenuation correction.
2. PET/CT and bone scan analysis will be conducted by two experienced nuclear medicine physicians with consensus interpretation.
3. PET - Anonymized PET scans will be analyzed with accompanying non- IV contrast CT, which is the standard of clinical care, but without knowledge of bone scan or CT results.
4. Bone Scans - Anonymized bone scans, coded differently from PET/CT scans, will be analyzed on a separate day from the PET/CT reading session. The bone scans will be analyzed with the accompanying CT scan available for anatomical correlation of bone scan findings, as is the standard of care. Prior two bone scans will be available for comparison to determine new, progressive, and stable disease.
5. Chest/abdomen/pelvis CT analysis will be conducted by an experienced radiologist without knowledge of bone scan or PET/CT results, as is also the standard of care.

Statistical Plan:
Index lesions on ^{18}F-DCFPyL scans will be scored based on SUVmax as previously described (53). We will correlate the uptake within these index lesions with radiographic response on CT or bone scan to determine if uptake in a given lesion might be associated with therapeutic response. The semi-quantitative measures of lesion uptake before and after BAT will be compared using paired –sample Wilcoxon signed rank tests. PSA and other serum markers in patients with positive or negative PET studies will be compared using the Mann-Whitney test.

Pharmaceutical Information

(Additional radiopharmaceutical information not listed below is downloaded on the FDA IND application in the supporting documents section of eIRB and available there for review).

Common Name: DCFPyL

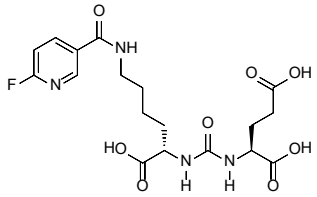
Chemical Name: (S)-2-(3-((S)-1-carboxy-5-(6-fluoronicotinamido)pentyl)ureido)pentanedioic acid

Characteristics: white powder

Chemical formula: $C_{18}H_{23}FN_4O_8$

C.A.S. number: 1423758-00-2

Structure:



Molecular Weight: 442.40

Solubility: DMSO, 6.7% ethanol in 0.9% sodium chloride, methanol, 1:1 acetonitrile:water

Initial Phase I study Adverse Events with ^{18}F-DCFPyL

An initial phase I study of the biodistribution and dosimetry of ^{18}F-DCFPyL was performed in nine men with metastatic prostate cancer..

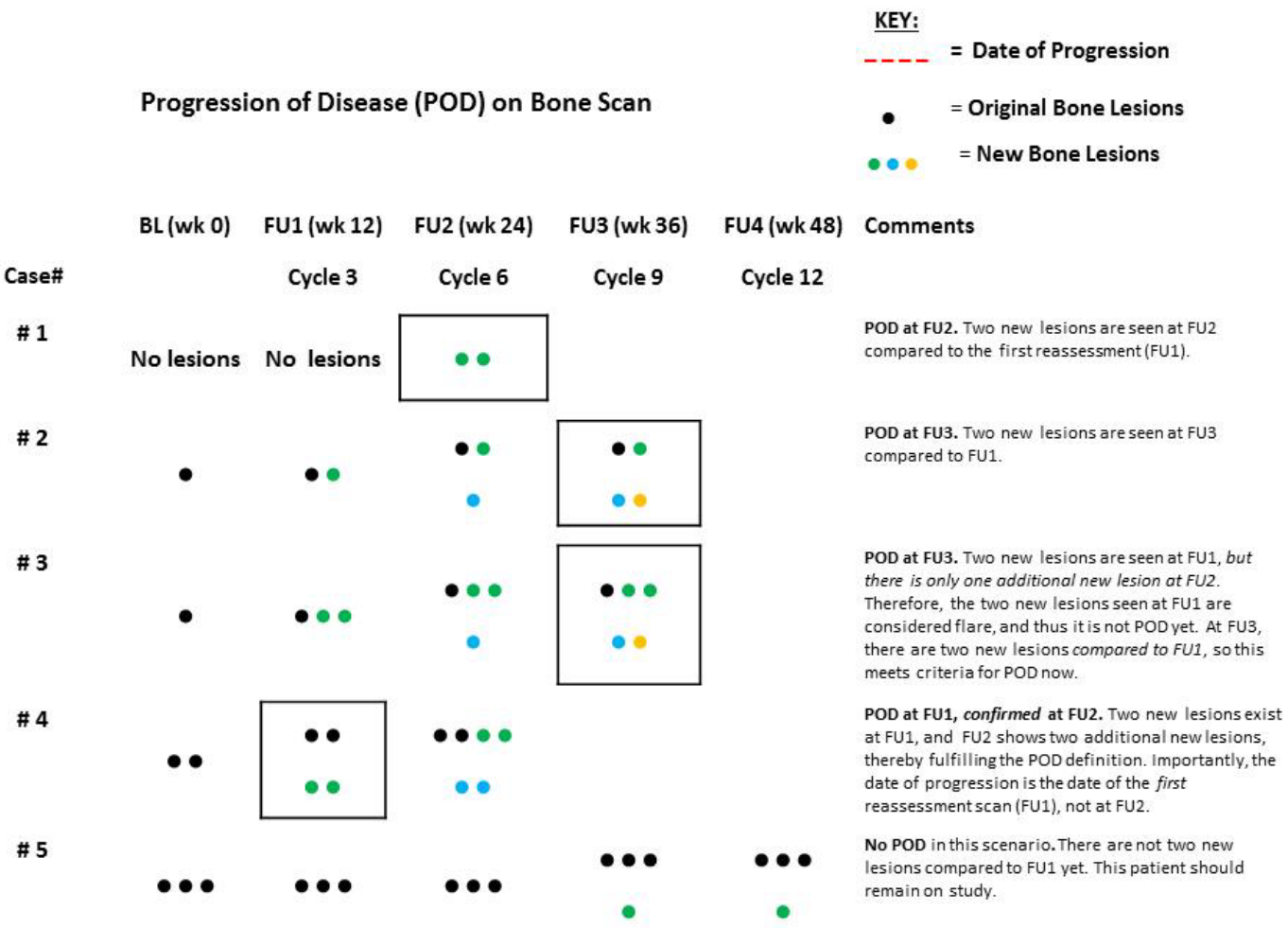
Participants did not experience any severe adverse events. One participant reported 2 adverse events that were classified as either unrelated or unlikely to be attributable to the radiopharmaceutical. Another participant experienced a grade 1 adverse event according to the Common Terminology Criteria for Adverse Events (National Cancer Institute). This participant experienced a decrease in platelet count on routine investigation assessment during the post imaging follow up which has not resolved. This is likely due to the participant starting prostate cancer treatment prior to the follow-up visit. There were no other adverse events reported.

Appendix 6

Data and safety monitoring will follow Level I under the SKCCC Data and Safety Monitoring Plan (DSMP, Version 5.0: 12/6/2012, NCI Approval Date: 12/11/2012).

Appendix 7

Assessment of Progression on Bone Scan



Appendix 8

Quality of Life Instruments