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Oncology

Supplementary appendix

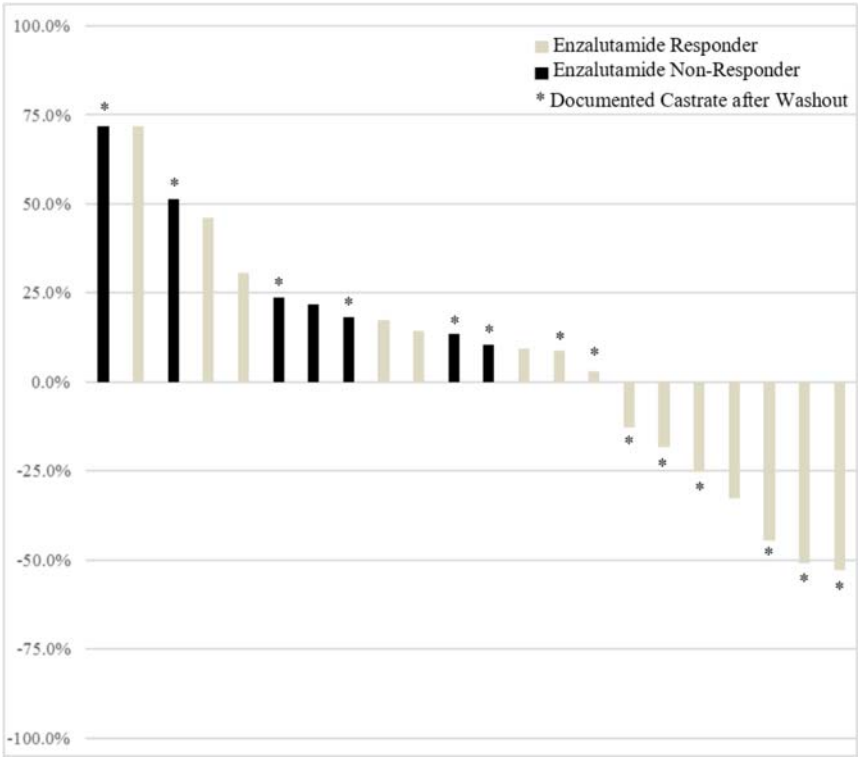
This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Teply BA, Wang H, Luber B, et al. Bipolar androgen therapy in men with metastatic castration-resistant prostate cancer after progression on enzalutamide: an open-label, phase 2, multicohort study. *Lancet Oncol* 2017; published online Dec 13. http://dx.doi.org/10.1016/S1470-2045(17)30906-3.

Supplementary Appendix

PSA changes after the 28-day washout period after BAT discontinuation.

Patients completing BAT were followed for a 28-day washout period to allow testosterone to fall to castrate range. A PSA was measured after 28-days, corresponding with first day of enzalutamide administration. This PSA served as the baseline for evaluation of enzalutamide response. Testosterone was also measured in the majority of patients after the 28-day washout.



PSA change after 28-day post-BAT washout. Patients going on to respond or not to respond to enzalutamide are noted. Patients who had a documented castrate testosterone after washout (<50ng/dl) are starred.

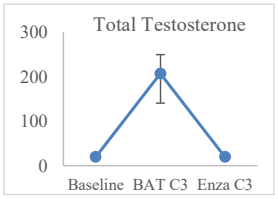
Adverse Events occurring on Enzalutamide.

Adverse Event	Grade 1-2, n (%)	Grade 3, n (%)	Grade 4, n (%)	Grade 5, n (%)
Musculoskeletal Pain*	6 (29%)	-	-	-
Fatigue	6 (29%)	-	-	-
Hypertension	3 (14%)	-	-	-
Anorexia	3 (14%)	-	-	-
Nausea	3 (14%)	-	-	-
Weight loss	3 (14%)	-	-	-
Cough	2 (10%)	-	-	-
Dizziness	2 (10%)	-	-	-
Dyspnea	2 (10%)	-	-	-
Hot Flashes	2 (10%)	-	-	-
Vomiting	2 (10%)	-	-	-
Abdominal pain	-	1 (5%)	-	-
Pancreatitis	-	-	1 (5%)	-
Paresthesias	-	1 (5%)	-	-
NSTEMI	-	1 (5%)	-	-
GI Bleed	-	1 (5%)	-	-

Those occurring in ≥10% of patients are listed for grades 1-2, and all events are listed for grades 3-5. *Musculoskeletal pain not deemed to be tumor flare or clinical progression.

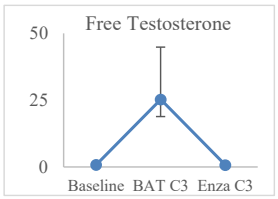
Metabolic parameters.

A



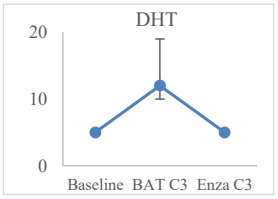
n=30 n=26 n=15
p<0.0001 p=0.59

B



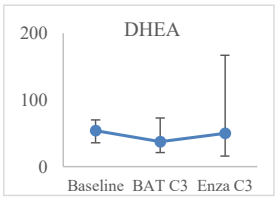
n=28 n=23 n=12
p<0.0001 p=0.68

C



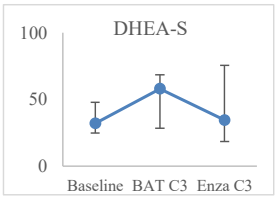
n=28 n=23 n=11
p<0.0001 p>0.99

D



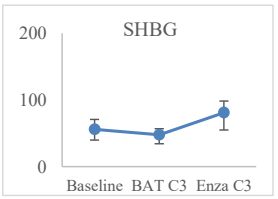
n=27 n=20 n=9
p=0.36 p=0.11

E



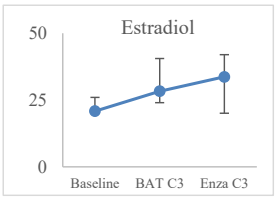
n=27 n=23 n=12
p=0.068 p=0.092

F



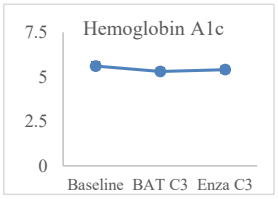
n=27 n=23 n=12
p=0.0006 p=0.0059

G



n=28 n=23 n=12
p=0.0013 p=0.042

H



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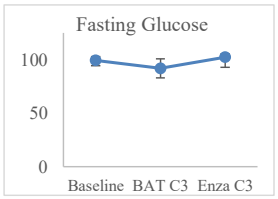
p=0.0066 p=0.10

I



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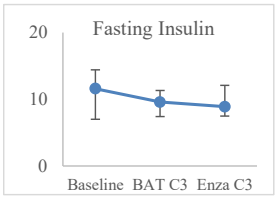
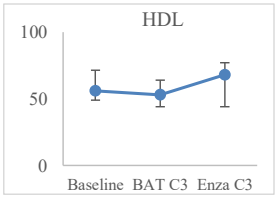
p=0.032 p=0.53

J



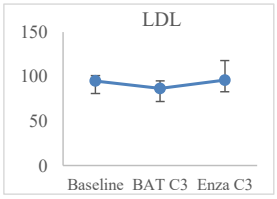
n=30 n=25 n=13
p=0.85 p=0.95

K



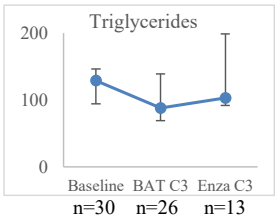
n=30 n=26 n=13
p=0.0014 p=0.83

L



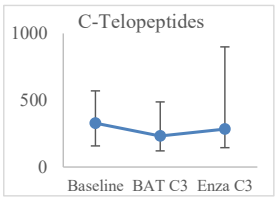
n=30 n=26 n=13
p=0.043 p=0.080

M



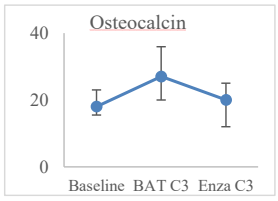
n=30 n=26 n=13
p=0.031 p=0.24

N



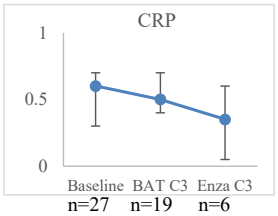
n=14 n=18 n=5
p=0.72 p=0.25

O



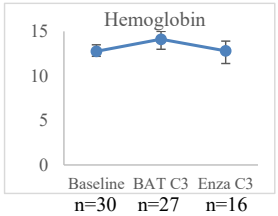
n=30 n=27 n=13
p<0.0001 p=0.62

P



n=27 n=19 n=6
p=0.65 p=0.58

Q



n=30 n=27 n=16
p=0.0074 p=0.84

Changes in metabolic parameters from baseline to after 3 cycles of BAT and Enzalutamide. All lab values were on D1 of cycle which corresponds to testosterone nadir each cycle. Data displayed are median values with 95% confidence intervals for (A) total testosterone, ng/dl; (B) free testosterone, pg/ml; (C) DHT, ng/dl; (D) DHEA, ng/dl); (E) DHEA-S, µg/dl; (F) SHBG, nmol/l; (G) estradiol, pg/ml; (H) hemoglobin A1c, %; (I) fasting glucose, mg/dl; (J) fasting insulin, mcU/ml; (K) HDL, mg/dl; (L) LDL, mg/dl; (M) triglycerides, mg/dl; (N) c-telopeptides, pg/ml; (O) osteocalcin, ng/ml; (P) CRP, mg/dl; (Q) hemoglobin, g/dl. Values below the lower limit of detection (such as DHT <5 ng/dl) are reported as at that limit. P-values are listed for the comparisons between baseline and BAT C3 and baseline and Enzalutamide C3 for each parameter.

A Phase II Study to Determine Sequential Response to Bipolar Androgen Therapy (BAT) followed by Enzalutamide or Abiraterone Post-BAT in Men with Prostate Cancer Progressing on Combined Androgen Ablative Therapies.

SYNOPSIS:

Title: A Phase II Study to Determine Sequential Response to Bipolar Androgen Therapy (BAT) followed by Enzalutamide or Abiraterone Post-BAT in Men with Prostate Cancer Progressing on Combined Androgen Ablative Therapies.

Objectives: The study has two primary objectives. The first objective is to determine if men with progressive metastatic CRPC post-castration only or post-treatment with enzalutamide or abiraterone acetate will exhibit a PSA response following cyclical administration of sufficient parenteral testosterone [i.e. Bipolar Androgen Therapy (BAT)] to achieve supraphysiologic serum testosterone levels. The second objective is to determine the PSA response to return to castrate levels of testosterone or retreatment with enzalutamide or abiraterone post-BAT as a strategy to re-sensitize CRPC cells and overcome resistance to androgen ablative therapies.

Study Design: Single-arm, single site, open label study of the effects of parenteral testosterone followed by enzalutamide, abiraterone or castration-only therapy in men with metastatic CRPC who previously progressed on one of these forms of therapy. The study will enroll three cohorts of patients: men with metastatic CRPC who have progressed on enzalutamide (Cohort A); men with metastatic CRPC who have progressed on abiraterone acetate (Cohort B); and men with metastatic CRPC who have progressed on first line castration-only therapy (Cohort C).

Treatment Plan: The trial will enroll up to 90 patients, 30 for each cohort. Eligible patients will continue on androgen ablative therapy with LHRH agonist (i.e. Zoladex, Trelstar, Eligard or Lupron) if not surgically castrated to suppress endogenous testosterone production. Patients will receive intramuscular injection with either testosterone cypionate or testosterone enanthate at a dose of 400 mg. This dose was selected based on data demonstrating that it produces an initial supraphysiologic serum level of testosterone (i.e. > 3-10 times normal level) with eugonadal levels achieved at the end of two weeks with return to near castrate levels by 28 days. Cycles are 28 days

in length. After 3 cycles (i.e. ~ 3 months) patients will have repeat PSA and bone/CT scans to establish the effect of BAT on these parameters.

Castrate Resistant Prostate Cancer Cohort:

The study will enroll a third cohort of patients: men with metastatic prostate cancer who have only received first line hormone therapy with LHRH agonist alone or LHRH agonist plus an anti-androgen. Patients who have developed castrate resistance to first line therapy and have then received second line hormone therapy of any kind (including flutamide, bicalutamide, nilutamide, ketoconazole, abiraterone, enzalutamide, ARN-509 and investigational anti-androgens) are not eligible for enrollment in this cohort (Cohort C).

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

1) Patients with PSA below pretreatment baseline and either objective response or stable disease on scans will receive additional courses of BAT until evidence of PSA or disease progression. Patient is considered a PSA responder if PSA is ≥50% below baseline level at any time on BAT.
2) Patients with a declining PSA that is at least 50% below the peak PSA level but not yet below baseline after 3 cycles will continue to receive BAT as long as PSA continues to decline from the previous value. Patient is considered a PSA responder if PSA is ≥50% below baseline level at any time on BAT.
3) A patient who's PSA begins to increase (as assessed after every 3 cycles) after an initial PSA decline that does not go below baseline PSA will not be considered a PSA responder. At time of PSA increase, that is ≥25% above previous PSA level, these patients will proceed to either enzalutamide or abiraterone acetate or be followed for response after serum testosterone has returned to a castrate level (Cohort C).
4) Patients with PSA that is not declining from peak levels and remains ≥25% above baseline will proceed to either enzalutamide or abiraterone acetate (Cohorts A and B) or be followed for response after serum testosterone has returned to a castrate level (Cohort C).

5) Patients who have had a PSA decline below baseline and then begin to have an increasing PSA and have stable CT/bone scans may remain on BAT at the treating physician's discretion if it is determined that the patient may be deriving clinical benefit from BAT. These patients may remain on BAT at physician's discretion until PSA increase above baseline. Time of PSA progression will be point at which patients met PCWG2 criteria for PSA progression.
6) Patients with radiographic progression on CT scan (per RECIST criteria) will stop BAT regardless of PSA response and will proceed to therapy with either enzalutamide or abiraterone acetate. Patients in Cohort C will stop BAT and remain on LHRH agonist therapy to return to castrate levels of testosterone.
7) Radiographic progression on bone scan will be defined by PCWG2 criteria as ≥ 2 new bone lesions. However, for the first reassessment scan only, patients with declining PSA 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 radiographic progression is the date of the first reassessment bone scan. If the confirmatory scan does not show any additional new lesions, patient remains on study. If progression is observed on subsequent bone scans, a confirmatory scan is not required; the date of this bone scan is the date of progression. Patients with bone scan progression based on these criteria will stop BAT regardless of PSA response and will proceed to therapy with either enzalutamide or abiraterone acetate. Patients in Cohort C will stop BAT and remain on LHRH agonist therapy to return to castrate levels of testosterone.

<u>Assessment of Response to enzalutamide (Cohort A), abiraterone (Cohort B) or castration-only (Cohort C) post-BAT (To occur after every 3 cycles):</u>

At time of progression on BAT, patients will remain on LHRH agonist alone for one month to re-establish a castrate level of T (<50 ng/dl). At this point they will begin treatment with enzalutamide at a dose of 160 mg p.o. per day or abiraterone acetate at a dose of 1000 mg p.o. per day. Patients on abiraterone acetate will also receive prednisone 5 mg p.o. bid. Cycles will be 28 days. Patients in first line castration-only Cohort C will remain on LHRH agonist therapy and receive no additional

androgen ablative hormonal therapy. Continued PSA Response will be assessed every cycle. Objective response will be assessed by repeat CT scan every 3 cycles and bone scan every 3 cycles.

1) PSA response is defined as a ≥50% PSA decrease below the post-BAT PSA level obtained at time of initiation of enzalutamide or abiraterone acetate or return to castrate levels of serum testosterone (Cohort C).
2) PSA progression is defined as a ≥25% PSA increase above the post-BAT PSA level over two successive measurements 4 weeks apart.
3) Patients with radiographic progression on CT scan (per RECIST criteria) will stop enzalutamide or abiraterone acetate regardless of PSA response. Patients on castration-only arm (Cohort C) with radiographic progression will remain on castration therapy but will come off study
4) Radiographic progression on bone scan will be defined by PCWG2 criteria as ≥ 2 new bone lesions. However, for the first reassessment scan only after treatment with abiraterone acetate or enzalutamide or return to first line castration-only therapy (Cohort C), patients with declining PSA should remain on study and have a confirmatory scan performed 12 weeks (3 cycles) later (Appendix 3). If this confirmatory scan shows 2 or more additional new lesions, this defines progression. The date of radiographic progression is the date of the first reassessment bone scan. If the confirmatory scan does not show any additional new lesions, the patient remains on study. If progression is observed on subsequent bone scans, a confirmatory scan is not required; the date of this bone scan is the date of progression. Patients with bone scan progression based on these criteria will stop enzalutamide or abiraterone acetate regardless of PSA response and will come off study. Patients on castration-only arm (Cohort C) with radiographic progression will remain on castration therapy but will come off study

Study Population: Men with asymptomatic metastatic CRPC with rising PSA who have been treated with continuous castrating androgen ablative therapy and have evidence of progression after enzalutamide, abiraterone acetate or first line castration-only therapy.

Number of Patients: 90

Inclusion criteria:

1. Performance status ≤2
2. Age ≥18 years
3. Histologically-confirmed adenocarcinoma of the prostate
4. Progressing on continuous androgen ablative therapy (either surgical castration or LHRH agonist)
5. Documented castrate level of serum testosterone (<50 ng/dl)
6. For Cohorts A and B, patients must have progressed on prior treatment with enzalutamide or abiraterone acetate + prednisone (by PSA criteria or radiographically).
7. For castration-only Cohort C, patients must have developed castrate resistant prostate cancer after progressing on first line hormone therapy with either surgical castration or LHRH agonist or LHRH agonist plus an anti-androgen.
8. Patients progressing on LHRH agonist plus an anti-androgen as first line therapy must be off anti-androgen for 4 weeks prior to first treatment with testosterone.
9. Patients with rising PSA on two successive measurements at least two weeks apart.
10. For Cohort A (enzalutamide) and Cohort B (abiraterone acetate)
    a. Prior treatment with up to 2 additional second line hormone therapies, including ketoconazole is allowed.
    b. Patients who have progressed on both enzalutamide and abiraterone acetate are eligible and post-BAT will be retreated with the last second line agent they had received (e.g. patient receiving abiraterone then enzalutamide would receive retreatment with enzalutamide post-BAT).
    c. Patients must be withdrawn from enzalutamide or abiraterone acetate for ≥ 4 weeks and have documented PSA increase after the withdrawal period.
    d. Patients receiving prednisone in conjunction with abiraterone acetate must be weaned off prednisone prior to starting BAT.
11. For Cohort C (castration-only):

a. Patients must continue on castrating therapy throughout BAT treatment.
b. No prior second line hormone treatment with flutamide, bicalutamide, nilutamide, enzalutamide, abiraterone, ketoconazole, ARN-509 or other investigational androgen ablative therapies is permitted for Cohort C.

12. 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.
13. Acceptable liver function:
    a. Bilirubin < 2.5 times institutional upper limit of normal (ULN)
    b. AST (SGOT) and ALT (SGPT) < 2.5 times ULN
14. Acceptable renal function:
    a. Serum creatinine < 2.5 times ULN, OR
15. 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.
16. At least 4 weeks since prior surgery with full recovery (no persistent toxicity ≥ Grade 1).
17. Ability to understand and willingness to sign a written informed consent document.

Exclusion criteria:

1. Pain due to metastatic prostate cancer requiring opioid analgesics.
2. > 5 sites of visceral disease in lung or liver (nonspecific lung nodules ≤1 cm in diameter are permitted).
3. Prior treatment with docetaxel or cabazitaxel for metastatic castration-resistant prostate cancer is prohibited.
4. Requires urinary catheterization for voiding due to obstruction secondary to prostatic enlargement thought to be due to prostate cancer or benign prostatic hyperplasia.

5. 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, spinal metastases with concern over spinal cord compression, lymph node disease with concern for ureteral obstruction).
6. 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.
7. Active uncontrolled infection, including known history of AIDS or hepatitis B or C.
8. Any psychological, familial, sociological, or geographical condition that could potentially interfere with compliance with the study protocol and follow-up schedule.
9. Prior history of a thromboembolic event within the past two years and not currently on systemic anticoagulation.
10. Hematocrit >50%, untreated severe obstructive sleep apnea, uncontrolled or poorly controlled heart failure [per Endocrine Society Clinical Practice Guidelines].

Study Endpoints:

Primary:

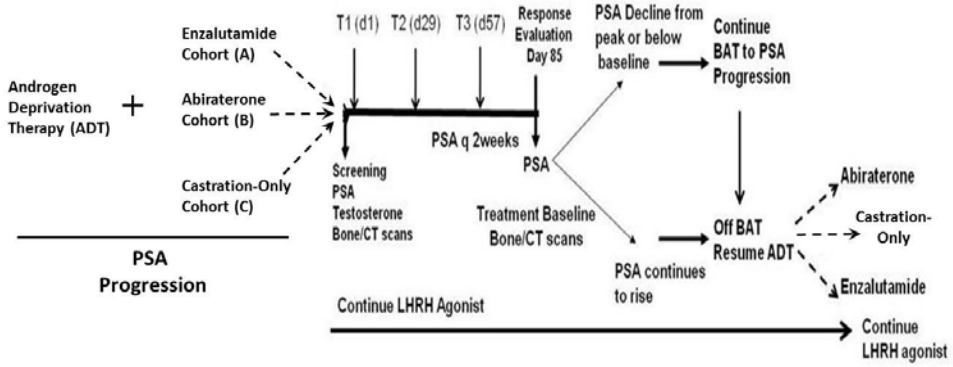
1. PSA response to BAT (≥50% PSA reduction from pre-BAT baseline level).
2. PSA response to enzalutamide or abiraterone acetate post-BAT (≥50% PSA reduction from baseline PSA level obtained at initiation of enzalutamide or abiraterone acetate post-BAT).
3. For Cohort C, PSA response to return to castrate levels of testosterone post-BAT.

Secondary:

1. Time to PSA progression on enzalutamide or abiraterone acetate or return to castration-only post-BAT.
2. Time to PSA progression on BAT.

3. Measurable disease response post-BAT and post-treatment with enzalutamide or abiraterone acetate or return to castration-only post-BAT.
    a. For soft tissue lesions, based on RECIST 1.1.
    b. For bone disease, based on PCWG2 criteria.
4. Time to initiation of docetaxel chemotherapy
5. Quality of life and metabolic studies
6. Effect of treatment with testosterone and abiraterone acetate or enzalutamide on bone uptake of Tc-99 MDP as assessed by serial quantitative Bone Scan with SPECT CT.
7. Safety and Tolerability
    a. Adverse events will be collected at each clinic visit and classified for severity according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.0. Determination of relationship of the adverse event to the study procedures or study drug will be performed by the PI using the standard attribution terminology (e.g. unrelated, possibly related, etc.).

Study Scheme:



Statistical Plan:

The joint primary objectives of the trial are to determine the PSA response to enzalutamide or abiraterone acetate post-BAT and the PSA response to BAT in men with metastatic CRPC who have progressed on enzalutamide or abiraterone acetate without prior treatment of chemotherapy. The study will enroll three cohorts of patients: men with metastatic CRPC who have progressed on enzalutamide (Cohort A); men with

metastatic CRPC who have progressed on abiraterone acetate (Cohort B); and men with metastatic CRPC who have progressed on first line castration therapy only (Cohort C). Each cohort will be evaluated separately. We wish to determine whether the rate of PSA response to re-treatment of enzalutamide or abiraterone or first line castration-only or the response to BAT exceeds 5%. The regimen will be considered active in regard to its ability to re-sensitize patients to enzalutamide or abiraterone acetate or castration-only if it results in a ≥25% PSA response rate to enzalutamide or abiraterone acetate or castration-only (i.e. an absolute 20% improvement compared to 5% response rate), and will be considered active in patients who have progressed on enzalutamide or abiraterone acetate or first line castration-only if it results in a ≥20% PSA response rate to BAT (i.e. an absolute15% increase compared to 5% response rate). The overall type I error will be 0.1, allocated between the two primary endpoints of PSA response to enzalutamide or abiraterone or first line castration-only (0.05) and PSA response to BAT (0.05).

The trial will enroll up to 90 patients, 30 for each cohort. Thirty evaluable patients will provide 90% power to reject the null 5% PSA response to enzalutamide/abiraterone acetate/first line castration-only in favor of 25% PSA response with a one-sided test at significance level 0.05. For the co-primary endpoint of PSA response to BAT, a total of 30 patients will provide 83% power to reject the null 5% PSA response to BAT in favor of 20% response to BAT. The patients who do not complete at least 3 cycles of BAT will be replaced.

One interim analysis and 1 final analysis are planned for PSA response to enzalutamide or abiraterone acetate or castration-only, and 1 final analysis are planned for PSA response to BAT. An interim analysis will be conducted to assess futility after PSA response to enzalutamide or abiraterone acetate or first line castration-only are available for the first 9 patients. According to the optimal Simon's two-stage design, if none of the patients respond to re-treatment with enzalutamide or abiraterone acetate or first line castration-only, the portion of the study testing re-treatment following progression on BAT will be terminated. At that point, enrollment will only continue to the

portion of the study evaluating BAT therapy. If there is at least 1 responder, the re-treatment portion of the study will continue and an additional 21 patients will receive re-treatment with enzalutamide or abiraterone acetate or first line castration-only therapy. If a total of 3 or fewer subjects achieve PSA response in stage one and two combined, we consider this regimen ineffective in its ability to re-sensitize patients to enzalutamide or abiraterone acetate or castration-only. If a total of 4 or more respond, we conclude the regimen is promising based on response to enzalutamide/abiraterone acetate/first line castration-only. For the response to BAT, if 4 or more responses are observed in the total of 30 patients, we conclude the regimen is promising based on response to BAT.

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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 deprive 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. This observation has led to a renewed interest in the AR axis as a therapeutic target.

Given that androgen signaling has been increasingly recognized as an important mediator of castration resistant prostate cancer (CRPC) cell growth, a number of “second-line” therapies aimed at further blocking androgen ligand signaling through AR have been under development. The culmination of this reasoning has been the development of the CYP17 inhibitor abiraterone acetate (Abi), an androgen synthesis inhibitor and enzalutamide (Enza), a potent antiandrogen. Both agents received FDA-approval based on modest survival benefit vs. placebo in men with metastatic CRPC

who had progressed on docetaxel chemotherapy (7,8). Abi was later approved for use in men with CRPC prior to docetaxel based on positive results from the COU-AA-302 trial. More recently, Enza has also been shown to be effective in the pre-docetaxel window, with preliminary results from the PREVAIL study demonstrating that it produces modest survival gains when given to docetaxel-naïve men. On the basis of this study, it is also expected to be approved for CRPC patients pre-chemotherapy in the near future.

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 Abi and Enza to LHRH agonist-based ADT in men with ADT progression. Unfortunately, resistance to these agents develops quickly. In addition, several small studies have demonstrated that sequential use of these agents in the post-chemotherapy setting is associated with significant reduction in PSA response, time to PSA progression and objective response suggesting cross-resistance between these agents, table 1 (9-12). 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 and emerging AR mutations that may affect Enza binding. Resistance first manifests as a sustained rise in the androgen responsive gene PSA, consistent with reactivation of a functioning AR axis.

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

	N=	Overall Survival (m)	>50% decline PSA (%)	TTP (m)	Objective Response (%)	Reference
Abiraterone Phase III (post-chemo)	1195	15.8	29.5	8.5	14	2
Abiraterone post Enzalutamide	38	7.2	8	2.7	8	4
Abiraterone post Enzalutamide	27	11.7	3	3.5	0	5
Enzalutamide Phase III (post-chemo)	1199	18.4	54	8.3	29	3
Enzalutamide post Abiraterone	35	7.1	28.6	4.9	2.9	6
Enzalutamide post Abiraterone	39	NR	12.8	2.8	4.3	7
Enzalutamide post Abiraterone	41	9.4	19.6	4.2	5.9	(unpublished)

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 through an auto-regulatory increase in expression of the full length AR and AR splice variants lacking the ligand binding domain (13-18). AR gene amplification is also

commonly seen in samples from patients on chronic androgen deprivation (19). These studies have demonstrated that this upregulation of AR may be responsible for the resistance to antiandrogens. It has also been shown that upon exposure to androgens (typically in the supraphysiologic range), an adaptively upregulated AR can become down regulated (14). This lowered AR expression can in turn re-sensitize CRPC cells to androgen ablative therapies such as anti-androgens.

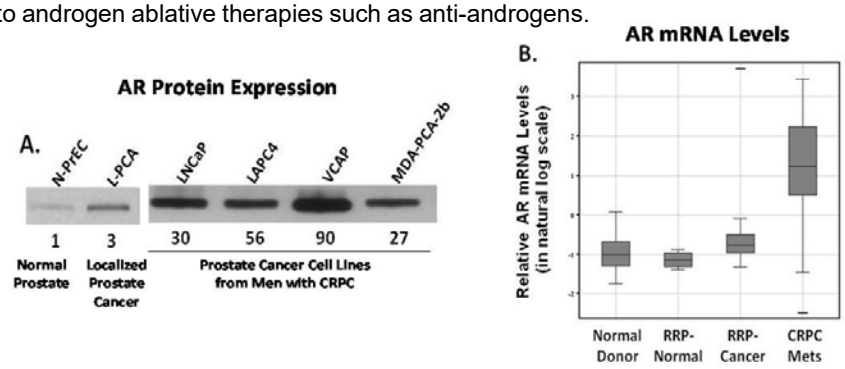


Figure 1. AR levels are elevated in human CRPC models and patient samples. (A) Relative expression of AR in normal prostate and ADT naïve prostate cancer compared to prostate cancer cell lines derived from men with CRPC. (B) mRNA expression of AR in ADT naïve prostate tissue vs. CRPC metastases (14).

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 addition of testosterone or other synthetic androgens to the media. Typically, in vitro data in human prostate cancer cell lines demonstrates a biphasic response to androgen 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 (20-22). 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 (23-25). 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, 26, 27). 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 (14, 26, 27). 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 protein synthesis, growth and loss of clonogenic survival (28). Finally, androgen starved cells upregulate both full length AR and variants of AR that are splice variants that cannot bind androgen due to loss of the ligand binding domain. 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 (29,30).

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 supraphysiologic to castrate levels of testosterone by treating men with intramuscular testosterone (31). 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

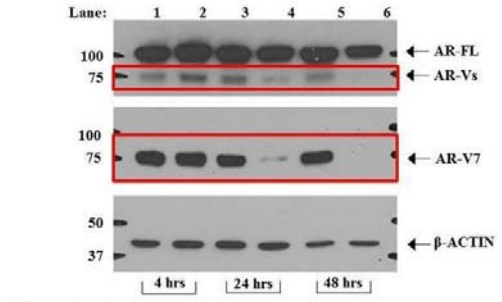


Figure 2. Effect of synthetic androgen on AR-Vs and AR-V7 in androgen deprived VCaP human prostate cancer cells

high androgen by dropping AR expression to low levels will then become sensitive to killing when testosterone levels drop. These cells with lowered AR levels will also become re-sensitized to treatment with the second line androgen ablative agents abiraterone acetate or enzalutamide.

1.2 Androgen Ablative Therapy for Prostate Cancer

The majority of men with prostate cancer that recurs outside of the prostate gland receive treatment at some point with therapies designed to lower serum testosterone to castrate levels (i.e. <50 ng/dl). These therapies include either surgical castration or medical castration with LHRH agonists. All men treated with standard castrating therapies eventually relapse and are classified as CRPC (3). This relapse initially manifests as an increasing serum PSA level in the setting of castrate levels of serum testosterone. With time, disease progression can be observed radiologically, typically manifesting as worsening or increasing numbers lesions seen on bone scan or CT scan. Eventually men with CRPC develop symptoms of bony pain and eventually succumb to the disease. Typically, when men are first classified as having CRPC and have rising PSA levels, they receive second line hormonal treatments. The rationale for the use of second-line hormone therapy relates to the observation that the adrenal gland, and perhaps the prostate cancer cells themselves, have the biochemical machinery necessary to synthesize testosterone from steroidal precursors (32). A variety of studies have documented that even in the castrate-resistant state, prostate cancer cells remain addicted to AR signaling for growth and survival. Castrate-resistant prostate cancer cells adapt to the low level circulating androgens through marked upregulation of AR expression. More recent studies have highlighted the importance of truncated AR splice variants that remain transcriptionally active despite lacking the ligand binding domain of the full length AR. Upregulation of these forms of AR ensures that sufficient transcriptionally active AR is present in the nucleus to support the ongoing growth and survival of these cells.

Up to this point, the treatment strategy for these prostate cancer cells that have become resistant to castrate levels of circulating androgens has been to attempt to disrupt AR signaling through the use of additional therapies aimed at disrupting ligand

binding to the AR. These therapies include antiandrogens and androgen synthesis inhibitors designed to non-testicular sources of androgen synthesis. Recently, two new agents, the testosterone synthesis CYP17 inhibitor abiraterone acetate and the antiandrogen enzalutamide were approved for the treatment of men with CRPC post-docetaxel based on survival advantage observed in Phase III studies (7,8). Abiraterone acetate has also been approved for men with CRPC prior to docetaxel therapy based on progression free survival advantage observed in a second Phase III study conducted in this population. It is expected that enzalutamide will be approved early in 2014 based on positive results from the PREVAIL study testing enzalutamide in men with CRPC in the pre-chemotherapy setting.

1.3 Preliminary Data in Support of Testosterone Therapy in CRPC

1.3.1 In Vitro Studies

The use of testosterone as treatment for men with CRPC receiving castrating therapies may at first seem paradoxical. However, significant in vitro and in vivo data has been generated previously to support this approach. Most of this data comes from studies using androgen sensitive human prostate cancer cell lines. Interestingly, all of the available androgen sensitive human prostate cancer cell lines were derived from men with CRPC. It has been known for some time that these “androgen sensitive” human prostate cancer cell lines have a biphasic response to androgen when grown in standard tissue culture media containing 10% fetal bovine serum (21-23). The addition of low levels of supplemental androgen to the culture media of these cells causes a slight increase in proliferation and PSA production. In contrast, higher levels of androgen, paradoxically, suppress growth of these so-called “androgen sensitive cells lines, figure 1a.

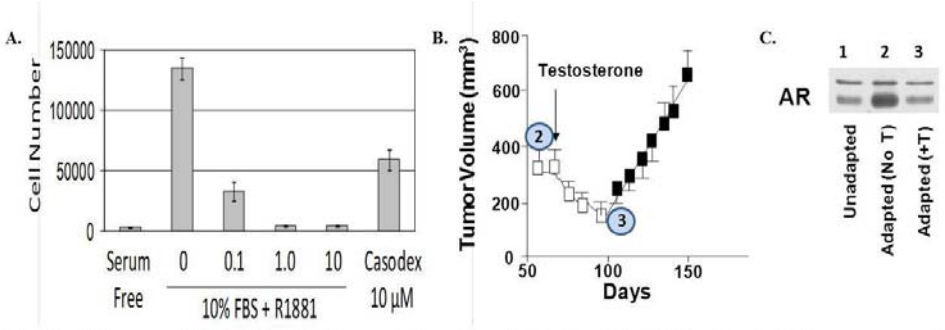


Figure 3. (A) Response of LNCaP cells adapted to grow in low androgen to treatment with R1881 or Casodex; (B) In vivo response of adapted to grow in low androgen to treatment with supraphysiologic testosterone; (C) AR expression levels in LNCaP cells at baseline (1), after adaptation to growth in low androgen (2) and after exposure to supraphysiologic testosterone over 100 days in vivo (3).

With serial passage in androgen deficient media, these human prostate cancer cells adapt and are able to grow with a higher proliferation rate in androgen deficient media compared to non-adapted cells. These adapted cells no longer increase proliferation rate in response to low level androgen and show marked decreases in proliferative rate following addition of low levels of androgen, figure 1a. This in vitro adaptation to low androgen conditions mimics the disease course in men with CRPC.

1.3.2 In Vivo Studies

In vivo studies using human prostate cancer xenografts have also supported this approach. In studies using the androgen adapted cell line described above, figure 3b, Chuu et al documented that xenografts of this line grew well in intact (i.e. non-castrated) nude mice in the absence of exogenous testosterone (25). However, treatment with testosterone via an implanted pellet increased blood testosterone to supraphysiologic levels (i.e.4-fold higher than testosterone level in intact mice) and resulted in rapid and sustained regression of tumors, figure 3b. Continued treatment with testosterone resulted in eventual tumor regrowth after ~ 100-day exposure.

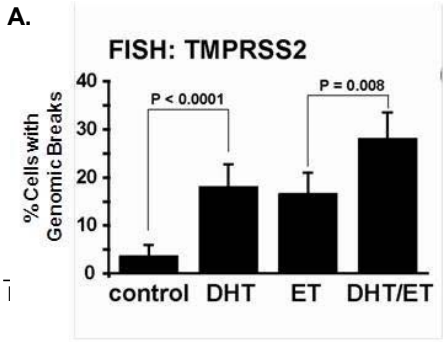
Analysis of tumor tissue from these various groups demonstrated that tumors formed from unadapted cells produced low level of androgen receptor (AR) growing under normal conditions in intact mice (lane 1, figure 3c). Adapted tumors that had been growing in vitro under conditions of decreased testosterone exhibit significant increase

in the level of AR message and protein, (lane 2 figure 3c) (13). This increase in AR level is similar to the increased levels of AR observed in human tumors from men with CRPC. In contrast, when the adapted tumors begin to grow once again under conditions of high testosterone, these tumors once again exhibit the lower levels of AR that are similar to baseline (lane 3, figure 3c).

1.3.3 Androgen Produces Double Strand Breaks in Human Prostate Cancer Cell Lines

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 Haffner et al suggests that one mechanism may involve the formation of androgen induced Topoisomerase II beta (TOP2B) mediated double strand breaks at AR target genes (28). 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 (figure 3a) 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 (figure 3 b, c).

A.



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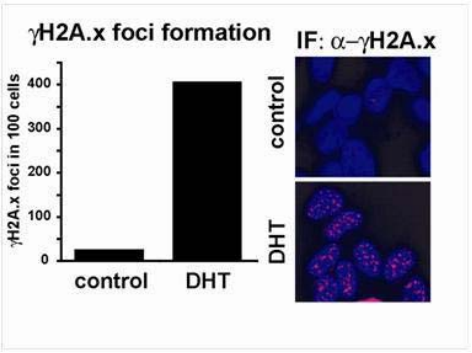


Figure 4. Androgen induced TOP2 mediated double strand breaks in prostate cancer cell lines stimulated with high levels of androgen. (A) Stimulation of androgen-deprived LAPC4 cells (control) with high levels of DHT (100 μM) alone leads to formation of genomic breaks at the TMPRSS2 gene in nearly 20% of cells. There is an additive effect on genomic break formation in cells treated with both DHT and ET suggesting a role of TOP2 in the formation of these breaks. (D) 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.

Interestingly, treatment of these cells with etoposide, a TOP2 poison that prevents enzymatic resolution of TOP2 induced double strand breaks, led to an additive effect on the formation of double strand breaks on these cells, suggesting that etoposide can be used to enhance double strand break formation in these cells. 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 3d). 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. In addition, these results suggest that the addition of etoposide to high dose testosterone can further enhance this growth suppression due to stabilization of the double strand breaks via inhibition of the TOP2B enzyme.

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

Another possible mechanism by which androgens may lead to prostate cancer growth suppression is through AR over-stabilization at DNA origins of replication (OR). Evidence that AR acts as a licensing factor in CRPC has been shown through studies demonstrating binding of the AR to replication complexes (RC) in proliferating CRPC cell lines (LNCaP, LAPC4, VCaP, and CWR22Rv1) figure 5A (14, 26, 27). Normally, the

AR is degraded via proteasomes after DNA replications and is not expressed in androgen sensitive (AS) prostate cancer cells undergoing mitosis (14, 26, 27). In the presence of supraphysiologic androgens, however, dividing AS prostate cancer cell lines will continue to express AR during mitosis (figure 5c,d). The presence of non-degraded AR going into subsequent cell cycles could lead to problems with DNA re-licensing. Further, there is evidence that cells with an over-expressed AR tend to exhibit cell cycle arrest in S-phase when exposed to androgens. This is what one would expect if a daughter cell were unable to re-license its DNA for replication (14, 26, 27).

As mentioned above, prostate cancer cells that are grown in an androgen deficient environment tend to be the most susceptible to the inhibitory effects of supraphysiologic androgens. Our group has shown that CRPC cells will adaptively auto-regulate the amount of AR expressed to >25-fold the normal level when in a chronically low androgen environment (14). This in theory would make them susceptible to AR over-stabilization at OR and possibly lead to problems with DNA re-licensing in subsequent cell cycles. Studies evaluating cell lines engineered to over-express the AR validate that increased AR expression makes them vulnerable to acute increases in androgen levels. This data provides a rational basis for alternating androgen deprivation therapy with supraphysiologic androgen replacement, termed bipolar androgen therapy (BAT). We validated the utility of BAT in a mouse LNCaP/A-cell xenograft model. In that model, castrated NOG mice were alternated between castrate and supraphysiologic androgen states through implantation and removal of a testosterone capsule. This lead to significant regression in tumor volumes (figure 5b) (14).

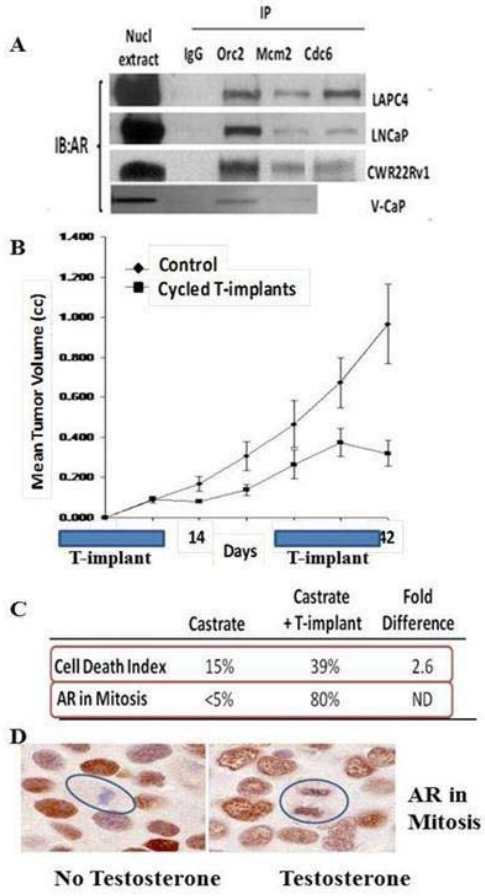


Figure 5: Rational for bipolar androgen therapy (BAT). (A) AR is a DNA-licensing factor. Blot shows result of co-immunoprecipitation studies showing that capture of AR with and anti-AR antibody pulls down a number of proteins associated with the origin of replication complex (Orc2, Mcm2, Cdc6). (B) Castrated 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). (C) Cell death index increase almost 3-fold in cells exposed to supraphysiologic T compared to control. Percent of cells exhibiting AR in mitotic figures markedly increases in BAT treated animals. (D) Example of AR staining in mitotic figure from BAT-treated tumor vs. control.

1.4 Clinical Experience with Testosterone in Prostate Cancer:

Up until recently, there had been 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 (33). 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 (34). In the long term

castrate in relapse group, 5 patients received testosterone for at least one month and 4 of 5 had subjective improvement. Five remaining patients received testosterone for 1-19 days and each had progression and came 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.

Two Phase I studies were reported describing the results of the use of testosterone gel as therapy for men with CRPC. In the first study, Szmulewitz et al evaluated the effect of increasing doses of transdermal testosterone in 15 men with early CRPC (rising PSA and minimal bone disease) (35). Five men each were treated with 2.5, 5.0 or 7.5 mg/day of transdermal testosterone which brought the median concentration of testosterone 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 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 testosterone 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 (36). They observed no grade 3 or 4 toxicities and no pain flares. Eugonadal serum testosterone levels were reported for this study. No objective responses were observed. Four patients had declines of PSA of at least 20% and one patient out of 12 achieved a ≥50% decline in PSA. Neither of the aforementioned studies achieved the supraphysiologic levels of testosterone that can be achieved with IM testosterone therapy. PSA declines were observed in some of the patients on these two studies, but only one patient out of 27 from the combined studies had a reported ≥50% decline in PSA.

On the basis of the combined results demonstrating the effects of supraphysiologic androgen on prevention re-licensing and the potential for production of stabilized DSBs in conjunction with etoposide, we designed a pilot clinical trial in which long term (<1

year) castrated men were given intramuscular injections of T in combination with 2 weeks dosing with oral etoposide (100 mg/day) (E) beginning with each new injection (37). IND exemption for use of testosterone in men with CRPC was obtained prior to initiating the study (IND 107,535). Eligible patients were required to have been on continuous ADT for ≥ 1 year with ≤ 5 sites of bone metastases and ≤ 10 sites of metastases overall. Sixteen men were enrolled and fourteen men [median age 69 (range 63-87)] with median baseline PSA of 20.1 ng/mL (range 1.4 to 819.1) completed at least 3 cycles of BAT and oral E. Prior therapies in these 14 men included: nilutamide or bicalutamide (n=9), Abi (n=2), ketoconazole (n=1) and Enza (n=1). At the end of 3 cycles, those patients demonstrating declining PSA were allowed to continue on BAT alone.

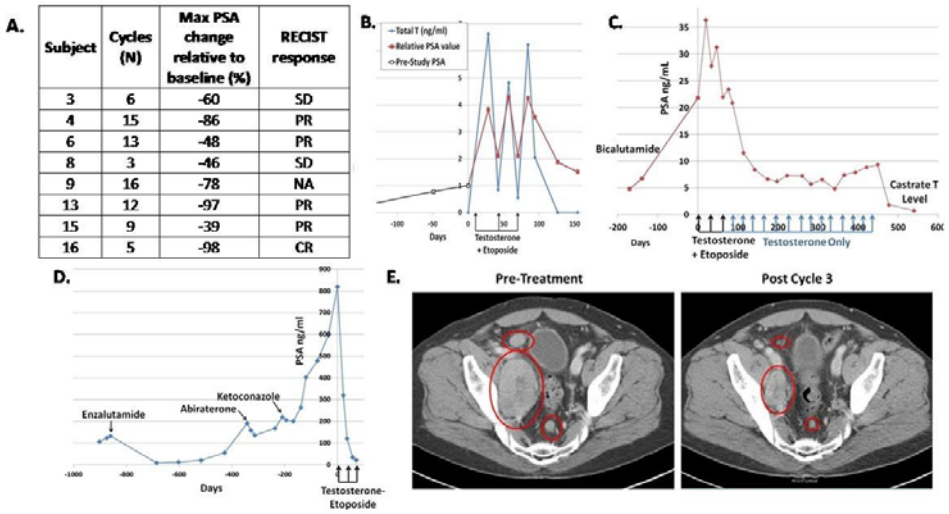
The FDA-approved dose of 400 mg T-cypionate (38) administered intramuscularly into the buttocks produced supraphysiologic serum T levels of >1500 ng/dL at 2 days post injection. Levels of T remained ~ 2-3 fold above physiologic levels for this age group at 2 weeks post-injection with average serum T levels of 694 ± 61 ng/dL at this time point. The average day 28 nadir T-level was 147± 29 ng/dL.

Overall the regimen was well-tolerated, table 2. Most adverse events were ≤ grade 2 and were primarily nausea, vomiting and fatigue (induced by the oral E). One patient developed neutropenia before completing the first cycle of E and died from sepsis and pneumonia. In contrast, minimal morbidity attributable to T treatment was observed. One patient developed grade 2 lower extremity edema that resolved with low dose diuretics. One patient developed priapism lasting >12 hours after one cycle of T and was removed from study. No patient experienced worsening of pain or obstructive urinary symptoms due to prostate cancer progression or tumor flare. Two patients were each found to have a single asymptomatic pulmonary embolus in a subsegmental vessel on the CT scan performed to assess response after 3 cycles. These emboli were thought to be “possibly related” to testosterone treatment.

Table 2. Comparison of Adverse Events across three studies of T in men with metastatic CRPC.

AE Possibly or Definitely Related to Testosterone	BAT Pilot (n=16)	Morris et al (n=12)	Szmulewitz et al (n=15)
Worsening Bone Pain	0	0	0
Urinary Obstruction	0	0	0
Skeletal Event	0	0	0
Edema (≤ grade 2)	7 (43%)	2 (15%)	2 (13%)
Mood Change	1 (6%)	0	0
Priapism	1 (6%)	0	0
Grade 3-4 AE	3 (21%)[a]	1 (8%)[b]	0

[a]Neutropenic sepsis and death attributable to etoposide (n=1)
[a]Asymptomatic pulmonary embolus (n=2)
[b]Cord compression in patient with known epidural disease and low back pain



Subject	Cycles (N)	Max PSA change relative to baseline (%)	RECIST response
3	6	-60	SD
4	15	-86	PR
6	13	-48	PR
8	3	-46	SD
9	16	-78	NA
13	12	-97	PR
15	9	-39	PR
16	5	-98	CR

Figure 6. Results from the pilot BAT study in men with CRPC. (A) Characterization of 8 patients with PSA response (B) PSA and serum T level in non-responder; (C) PSA response in patient receiving 3 cycles of T+ E and then 15 additional cycles of T alone. Patient demonstrated renewed sensitivity to ADT after progressing on BAT; (D) Patient with 98% decrease in PSA after 3 cycles of T+E after progression to enzalutamide, abiraterone and ketoconazole; (E) Patient demonstrating >50% decrease in size of pelvic lymph nodes after 3 cycles of T+E.

Eight of the fourteen patients (57%; 95% confidence interval 31-79%] who completed the initial 3 cycles of BAT + E experienced PSA declines, figure 6A. Of the 10 men with RECIST-evaluable disease, 2 had progressive (PD) and 3 had stable disease (SD), 4 had partial (PR) and 1 had a complete response (CR) for an objective response rate of 50%. Median response duration was 248 D (range, 80 to 359 D). The median time on study for the 8 patients demonstrating PSA response was 12 cycles (range 3-18). Four patients have remained on therapy ≥ 12 cycles (12, 13, 15, 18 cycles). Two patients remain on study with PSA declining by 98% and 36% after 8 and 12 cycles respectively. Independent of PSA-response, following completion of BAT 8/9 (90%) men have had a PSA decline to subsequent second line androgen ablative therapies (4/5 abiraterone acetate, 2/2 enzalutamide, 2/2 bicalutamide or nilutamide). While formal QoL testing was not performed in this pilot study, once completing the 3 cycles E, long term responders on T alone noted subjective improvement in energy level, sense of well-being, strength and vitality. Patients who were impotent due to ADT rapidly regained ability to have erections.

The lessons learned from this pilot trial were:

A) BAT could be administered safely to men on chronic ADT with moderate burden metastatic CRPC without producing worsening signs or symptoms due to prostate cancer progression.

B) Evaluation of PSA response in these patients is complicated by Supra-T stimulation of PSA production.

C) Despite initial PSA stimulation, Supra-T produces sustained PSA declines in some men with CRPC.

D) The sustained PSA response of many patients on subsequent cycles of BAT alone suggested that etoposide may not add anything to the response but did produce significant toxicity.

E) Following the first T injection 12 of 14 patients had an initial spike in PSA up to as high as 10-fold above baseline suggesting that these CRPC cells must continue to maintain a functional AR axis.

In addition to the pilot study of BAT + etoposide, we are currently performing a second single site open label study at Johns Hopkins evaluating the safety and efficacy

of alternating therapy with ADT and BAT. In this Phase II trial entitled “Bipolar Androgen Therapy in Men with Androgen-ablation Naïve Prostate Cancer (BATMAN)” patients receive a 6-month lead in of ADT followed by 3 monthly injections of Supra-T alternating with 3 months of ADT. The primary endpoint of this study is disease response as determined by the percent of subjects with a PSA response and/or radiographic response at the end of the study period. Secondary endpoints include safety, QOL and effects of treatment on metabolic parameters. This trial opened for accrual at Johns Hopkins in January of 2013. Thus far, 31 out of a total 31 men have been enrolled in a little over one year. Twenty patients have begun the BAT injections without any significant adverse effects (e.g. increased pain).

Therefore, while there is a theoretical risk of producing a tumor “flare” phenomenon upon administering supraphysiologic doses of testosterone to men with metastatic prostate cancer, we have not observed this in practice. In fact, the opposite has been true, with a sizable proportion of men actually showing objective evidence of disease response following testosterone based therapy.

BAT may overcome therapeutic resistance by re-sensitizing CRPC cells to androgen ablative therapies:

Multiple lines of evidence have established that AR overexpression may be the key molecular determinant of castration resistance and resistance to antiandrogens. Prostate cancer cells have the ability to autoregulate AR expression as an adaptive response to androgen levels in the tumor environment. Data from our lab and others suggests that just as human prostate cancer cells can upregulate AR in response to chronically low androgen environment, they can also downregulate AR levels when androgen levels are increased. More recent data suggests a role for ligand independent truncated AR-Vs in resistance to ADT, Abi and Enza (9-12). Knockdown of these AR-Vs by siRNA can re-establish sensitivity to antiandrogens such as bicalutamide and Enza (15, 16). Data from our group and others have documented that exposure to supraphysiologic androgen is an alternative way to eliminate or lower full-length AR levels in CRPC cells, figure 2. (29, 30).

These preclinical results suggest that treatment of CRPC cells with BAT has the potential to restore sensitivity to subsequent androgen ablative therapies. Analysis of the men treated in our pilot study shows that following PSA progression in patients who initially had PSA response to BAT and in patients who did not, 10/12 (83%) of these patients had a renewed PSA response to either ADT, antiandrogens, or Abi suggesting that BAT may have "re-sensitized" these CRPC cells to ADT therapies. Although the number of patients is small, 7/12 (58%) patients had sustained drops in PSA of several months duration once they returned to castrate serum T-levels. 2/2 patients who entered the study progressing on the anti-androgen bicalutamide had sustained drops in PSA when they were rechallenged with this agent after BAT. 4/5 (80%) patients who received Abi after BAT had ≥50% decrease in PSA including 3 patients who had progressed on prior ketoconazole. 2/2 patient treated with Enza post BAT also had marked decrease in PSA levels of >80% after one month of Enza therapy. One of these patients had progressed on bicalutamide, nilutamide and ketoconazole prior to BAT and had a 99% PSA decline within one month of Enza therapy post-BAT, figure 7, with a CT scan that showed "Substantial interval improvement from prior exam, with resolution of supraclavicular, mediastinal and retroperitoneal adenopathy…", figure 7C. These observations form the basis for this trial to determine if BAT can re-sensitize CRPC cells to retreatment with Enza or Abi.

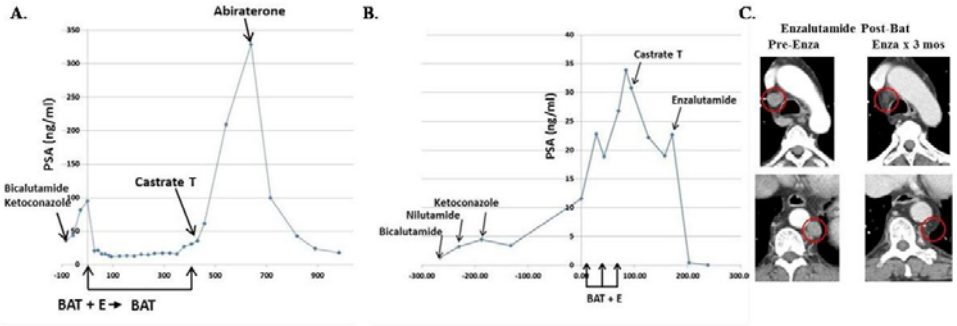


Figure 7. BAT may re-sensitize to ADT. (A) Patient refractory to ADT, bicalutamide and ketoconazole remains on BAT for 15 cycles then shows PSA progression on ADT followed by PSA decline of 70% at 3 mos and 95% at 1 yr of Abi. (B) Patient progressed on 2 antiandrogens and ketoconazole with no PSA decline to BAT shows PSA decline on return to ADT and then a 99% drop in PSA level after 1 mos on Enza. (C) Complete response observed in lymph nodes in this patient.

2. STUDY OBJECTIVES

2.1 Primary Objectives

2.1.1 Primary:

1. PSA response to BAT (≥50% PSA reduction from pre-BAT baseline level).
2. PSA response to enzalutamide or abiraterone acetate or first line castration-only therapy post-BAT (≥50% PSA reduction from baseline PSA level obtained at initiation of enzalutamide or abiraterone acetate or first line castration-only therapy post-BAT).

2.2. Secondary Objectives

2.2.1 Time to PSA progression on enzalutamide or abiraterone acetate or first line castration-only therapy post-BAT.

2.2.2 Time to PSA progression on BAT.

2.2.3 Measurable disease response post-BAT and post-treatment with enzalutamide or abiraterone acetate or first line castration-only therapy post-BAT.

a. For soft tissue lesions, based on RECIST 1.1.

b. For bone disease, based on PCWG2 criteria.

2.2.4 Time to initiation of docetaxel chemotherapy.

2.2.5 Quality of Life Effects (FACIT-F, RANDSF-36, Brief Pain Inventory, IIEF, PANAS) and metabolic studies.

2.2.6 Safety and Tolerability

a. Adverse events will be collected at each clinic visit and classified for severity according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.0. Determination of relationship of the adverse event to the study procedures or study drug will be performed by the PI using the standard attribution terminology (e.g. unrelated, possibly related, etc.).

3. PATIENT POPULATION AND SELECTION

The study will enroll three cohorts of patients: men with metastatic CRPC who have progressed on enzalutamide (Cohort A); men with metastatic CRPC who have progressed on abiraterone acetate (Cohort B); and men with metastatic CRPC who have progressed on first line castration-only (Cohort C). The trial will enroll up to 90 patients, 30 for each cohort.

3.1 Inclusion Criteria

1. Performance status ≤2
2. Age ≥18 years
3. Histologically-confirmed adenocarcinoma of the prostate
4. Progressing on continuous androgen ablative therapy (either surgical castration or LHRH agonist)
5. Documented castrate level of serum testosterone (<50 ng/dl)
6. For Cohorts A and B, patients must have progressed on prior treatment with enzalutamide or abiraterone acetate + prednisone (by PSA criteria or radiographically).
7. For castration only Cohort C, patients must have developed castrate resistant prostate cancer after progressing on first line hormone therapy with either surgical castration or LHRH agonist or LHRH agonist plus an anti-androgen.
8. Patients progressing on LHRH agonist plus an anti-androgen as first line therapy must be off anti-androgen for 4 weeks prior to first treatment with testosterone.
9. Patients with rising PSA on two successive measurements at least two weeks apart.
10. For Cohort A (enzalutamide) and Cohort B (abiraterone acetate)
    a. Prior treatment with up to 2 additional second line hormone therapies, including ketoconazole is allowed.
    b. Patients who have progressed on both enzalutamide and abiraterone acetate are eligible and post-BAT will be retreated with the last second line agent they had received (e.g. patient receiving abiraterone acetate then enzalutamide would receive retreatment with enzalutamide post-

BAT).

c. Patients must be withdrawn from enzalutamide or abiraterone acetate for ≥ 4 weeks and have documented PSA increase after the withdrawal period.
d. Patients receiving prednisone in conjunction with abiraterone acetate must be weaned off prednisone prior to starting BAT.

11. For Cohort C (first line castration-only):
    a. Patients must continue on castrating therapy throughout BAT treatment.
    b. No prior second line hormone treatment with flutamide, bicalutamide, nilutamide, enzalutamide, abiraterone acetate, ketoconazole, ARN- 509 or other investigational androgen ablative therapies is permitted for cohort C.
12. 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.
13. Acceptable liver function:
    a. Bilirubin < 2.5 times institutional upper limit of normal (ULN)
    b. AST (SGOT) and ALT (SGPT) < 2.5 times ULN
14. Acceptable renal function:
    a. Serum creatinine < 2.5 times ULN, OR
15. 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.
16. At least 4 weeks since prior surgery with full recovery (no persistent toxicity ≥ Grade 1).
17. Ability to understand and willingness to sign a written informed consent document.

3.2 Exclusion Criteria

1. Pain due to metastatic prostate cancer requiring opioid analgesics
2. >5 sites of visceral disease in lung or liver (nonspecific lung nodules ≤1 cm in diameter are permitted).

3. Prior treatment with docetaxel or cabazitaxel for metastatic castration-resistant prostate cancer is prohibited.
4. Prior seizures while on enzalutamide therapy.
5. Requires urinary catheterization for voiding due to obstruction secondary to prostatic enlargement thought to be due to prostate cancer or benign prostatic hyperplasia.
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, spinal metastases with concern over spinal cord compression, lymph node disease with concern for ureteral obstruction).
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 AIDS or hepatitis B or C.
9. Prior history of a thromboembolic event within the last two years and not currently on systemic anticoagulation.
10. Prior myocardial infarction within one year of study entry.
11. Hematocrit >50%, untreated severe obstructive sleep apnea, uncontrolled or poorly controlled heart failure [per Endocrine Society Clinical Practice Guidelines (67)].
12. Any psychological, familial, sociological, or geographical condition that could potentially interfere with compliance with the study protocol and follow-up schedule.

3.3 Inclusion of women and minorities

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

4. TREATMENT PLAN

4.1 Study Design

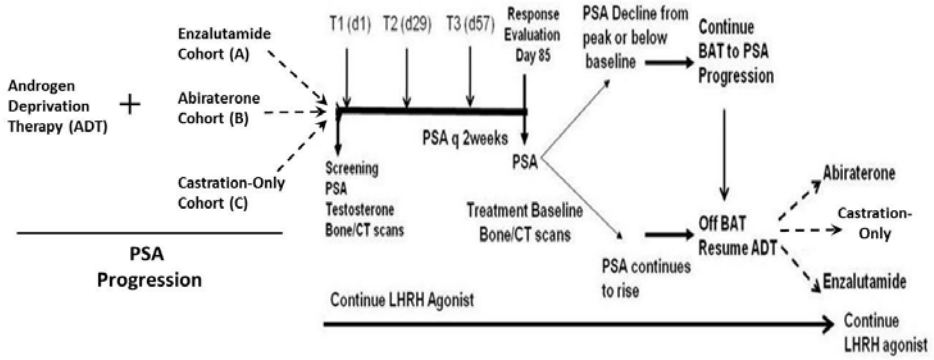
This is a single-arm, single site, open label study of the effects of parenteral testosterone followed by enzalutamide or abiraterone acetate or first line castration-only therapy in men with metastatic CRPC who previously progressed on either of these agents.

Treatment Plan: Eligible patients will continue on androgen ablative therapy with LHRH agonist (i.e. Zoladex, Eligard, Trelstar or Lupron) if not surgically castrated to suppress endogenous testosterone production. Patients will receive intramuscular injection with either testosterone cypionate or testosterone enanthate at a dose of 400 mg. This dose was selected based on data demonstrating that it produces an initial supraphysiologic serum level of testosterone (i.e. > 3-10 times normal level) with eugonadal levels achieved at the end of two weeks with return to near castrate levels by 28 days. Cycles are 28 days in length. After 3 cycles (i.e. ~ 3 months) patients will have repeat PSA and bone/CT scans to establish the effect of BAT on these parameters. Patients will continue on subsequent BAT cycles based on PSA response as described in section 6.1 “Assessing PSA Response to BAT”.

At time of progression on BAT, patients will remain on LHRH agonist alone for one month to re-establish a castrate level of T (<20 ng/dl). PSA obtained at time of return to castrate T will be the new baseline level to assess re-sensitization to enzalutamide or abiraterone acetate. At this point those patients who began the study after enzalutamide progression (Cohort A) will begin treatment with enzalutamide at a dose of 160 mg p.o. per day and those with progression on abiraterone acetate (Cohort B) at study entry will receive abiraterone acetate at a dose of 1000 mg p.o. per day with Prednisone 5 mg p.o. bid. Patients with prior liver toxicity with abiraterone acetate will be re-treated with last dose level not associated with liver toxicity. Those patients who began study after first line castration-only therapy (Cohort C) will remain on LHRH agonist therapy only and receive no additional androgen ablative therapy post-BAT. Cycles will be 28 days. Continued PSA response will be assessed every cycle. Objective response will be assessed by repeat CT and bone scan every 3 cycles. PSA response will be

determined as described in section 6.2 “Assessing PSA Response to enzalutamide or abiraterone acetate or castration-only therapy”.

4.2 Treatment Scheme:



4.3 Dosing Delays and Modifications

Treatment will be given on indicated days ± 2 days.

4.4 Removal of Patients from Study

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

1. Evidence of progression based on assessment of PSA response or measurable disease progression
   - Unacceptable adverse event(s) Patients develop urinary outlet obstruction thought to be due to prostate cancer within the prostate and requiring urinary catheterization
   - Patients who develop grade 3 or higher liver function abnormalities with increase in bilirubin, AST (SGOT) or ALT (SGPT) ≥ 2.5 times institutional upper limit of normal (ULN)
   - Patients develop decreased renal function with serum creatinine ≥ 2.5 times baseline level

- Patents develop hypersensitivity or anaphylactoid reactions to testosterone injection.
- Patients develop nausea/vomiting that cannot be controlled with oral antiemetic regimen.
- Intercurrent illness that prevents further participation

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

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

Due to the nature of the treatment, there is concern that patients could experience potential development of prostate cancer related pain due to 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, table 2. 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 PSA response. However, if patients have unequivocal clinical progression without PSA progression, these patients are indicated for the current standard of care. Study treatment should be

stopped and patients advised regarding available treatment options. For this study, unequivocal clinical progression will be characterized as:

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

4.6 Pain management:

Patients who develop new pain thought by the investigator to be due to prostate cancer should be treated according to NCCN guidelines for adult Cancer Pain (Ver. 2.2013) in a stepwise fashion using the WHO Analgesic Ladder approach. Patients with pain that is managed without oral opioids after 3 weeks can continue on to the next cycle of the study. Patients with a continued requirement for oral opioids after 3 weeks will be removed from study. Patients who have recurrent pain requiring oral opioids on subsequent cycles will also be removed from study. Patients with sustained or escalating pain after 3 weeks may be treated with daily bicalutamide at a dose of 150 mg at the investigator's discretion.

5. TREATMENT ASSESSMENT AND EVALUATION

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

5.1 Screening Studies (performed within 28 days before enrollment)

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. Inflammation Panel (C Reactive Protein)
11. Baseline EKG
12. Partial Prothrombin (PTT) and Prothrombin Times (PT)
13. Staging imaging with CT
14. Staging imaging with bone scintigraphy (bone scan with SPECT CT) (within 8 weeks before enrollment)
15. Blood drawn for research testing to assess level of full length and variant androgen receptor
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)

5.2 Treatment Period

1. Clinic visits every cycle for assessment of toxicity, ECOG performance status, vital signs and body composition.
2. PSA every cycle.

3. CBC, Comp Panel, at B2, B4 and every 3 months in BAT responders and at PB4 and every 3 months in in those responding to either Enzalutamide or Abiraterone acetate + Prednisone or Castration-only therapy.
4. Lipid Panel (fasting), Androgen Panel, Metabolic Panel, Inflammation Panel, PT/PTT at every 3 months x 3 while on BAT and every 3 months x 2 while on Enzalutamide/Abiraterone acetate/Castration-only therapy.
5. Standard CT scan and Bone scan with SPECT CT at B4.
6. BAT responders will then undergo CT scan and conventional planar whole-body bone scan (i.e. standard bone scan) every 3 months and at PB4
7. At PB4 visit, patients on Enzalutamide or Abiraterone acetate or castration-only therapy will have standard CT scan in addition to Bone Scan with SPECT CT. Those responding to either Enzalutamide or Abiraterone acetate + Prednisone or castration-only therapy will then have standard CT scan and standard bone scan every 3 months.
8. Blood will be drawn for research testing to assess level of full length and variant androgen receptor at C1 of BAT day 85 and then again at post–BAT after 3 cycle on Enzalutamide or Abiraterone acetate or castration only therapy (PB Cycle 1 Day 85).
9. Quality of life surveys at B4 and every 3 months X3 in BAT responders and at PB4 and every 3 months X 2 in in those responding to either Enzalutamide or Abiraterone acetate + Prednisone or castration only therapy.

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

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

6. STUDY ASSESSMENTS

6.1 Assessing 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 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) Patients with PSA below pretreatment baseline (PSA Response to BAT, figure 8A) and either objective response or stable disease on scans will receive additional courses of BAT until evidence of PSA progression (≥25% increase of PSA above nadir value). These patients will be considered PSA responders if PSA declines ≥50% below baseline at any time while on BAT.
2) Patients with declining PSA (i.e. lower than peak PSA level) that is not yet below baseline after 3 cycles (PSA decline, figure 8B) will continue to receive BAT as long as PSA continues to decline from the previous value. These patients will be considered PSA responders if PSA declines ≥50% below baseline in subsequent cycles of BAT.
3) Patients whose PSA initially declines from peak levels but then begins to increase prior to decreasing below baseline will not be considered PSA responders. At time of PSA increase that is ≥25% above previous PSA level, these patients will proceed to either enzalutamide or abiraterone acetate or castration-only therapy.
4) Patients with PSA that is not declining from peak levels and remains above baseline will proceed to either enzalutamide or abiraterone acetate or castration-only therapy (PSA Progression, figure 8C).
5) Patients exhibiting PSA levels that rise and fall with testosterone level (i.e. pattern in figure 8D) that are ≥25% above baseline with no sustained decline from peak PSA levels will be considered non-responders and will also proceed to treatment with Enzalutamide or Abiraterone or castration-only therapy.
6) Those patients responding to BAT will continue on BAT until progression, which is defined as an increase in PSA of ≥25% above the nadir value and confirmed by a repeat PSA 4 weeks later (PCWG2 definition).

7) Patients who have PSA progression but have stable CT/bone scans may remain on BAT at the treating physician's discretion if it is determined that the patient may be deriving clinical benefit from BAT. Time of PSA progression will be point at which patients first met PCWG2 criteria for PSA progression.
8) Patients with radiographic progression on CT scans by RECIST criteria or bone scans by PCWG2 criteria described below will come off BAT regardless of PSA response and proceed to enzalutamide or abiraterone acetate or castration-only therapy.

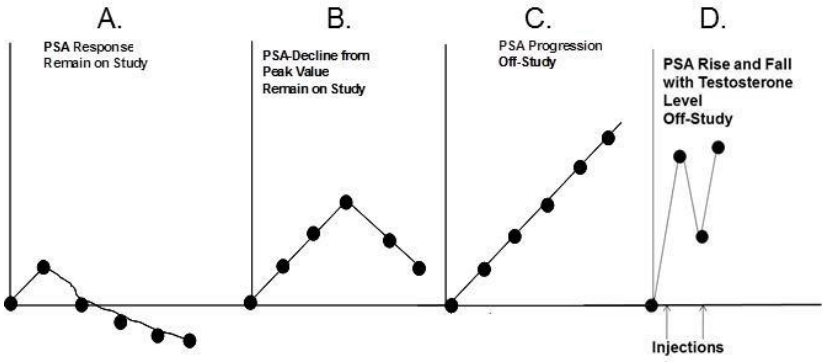


Figure 8. Examples of PSA response on study consistent with (A) PSA Response; (B) PSA-Decline; (C) PSA-Progression; (D) PSA tracking with serum testosterone level

6.2 Assessing PSA Response to Enzalutamide or Abiraterone or Castration-Only therapy post-BAT:

At time of progression on BAT, patients will remain on LHRH agonist alone for one month to re-establish a castrate level of T (<50 ng/dl). PSA obtained at time of return to castrate T will be the new baseline level to assess re-sensitization. At this point those patients who began the study after enzalutamide progression (Cohort A) will begin treatment with enzalutamide at a dose of 160 mg p.o. per day and those with progression on abiraterone acetate (Cohort B) at study entry will receive abiraterone acetate at a dose of 1000 mg p.o. per day with Prednisone 5 mg p.o. bid. Patients with

prior liver toxicity with abiraterone acetate will be re-treated with last dose level not associated with liver toxicity. Patients who began the study after first line castration-only progression (Cohort C) will continue on castrating therapy (i.e. LHRH agonist if not surgically castrated). Cycles will be 28 days. Continued PSA response will be assessed every cycle. Objective response will be assessed by repeat CT and bone scan every 3 cycles.

1) PSA response is defined as a ≥50% PSA decrease below the post-BAT PSA level obtained at time of initiation of enzalutamide or abiraterone acetate + prednisone or castration-only therapy.
2) PSA progression is defined as a ≥25% PSA increase above the post-BAT PSA level over two successive measurements 4 weeks apart.
3) Radiographic progression is defined as evidence of new lesions on bone scan (per PCWG2 criteria below) or progression on CT scan (per RECIST criteria) compared to baseline studies.

6.3 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 or Abiraterone or return to castration-only treatment. Progression for soft tissue lesions will be based on RECIST 1.1 criteria and for bone lesions based on PCWG2 criteria.

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

Patients with radiographic progression on CT scan (per RECIST criteria) will stop BAT regardless of PSA response and will proceed with either enzalutamide or abiraterone acetate or castration-only therapy.

Radiographic progression on bone scan will be defined by PCWG2 criteria as ≥ 2 new bone lesions. However, for the first reassessment scan only, patients with declining PSA should remain on study and have a confirmatory scan performed 12 weeks (3 cycles) later (Appendix 3). If this confirmatory scan shows 2 or more additional new lesions, this defines progression. The date of radiographic progression is the date of

the first reassessment bone scan. If the confirmatory scan does not show any additional new lesions, patient remains on study. If progression is observed on subsequent bone scans, a confirmatory scan is not required; the date of this bone scan is the date of progression. Patients with bone scan progression based on these criteria will stop BAT regardless of PSA response and will proceed with either enzalutamide or abiraterone acetate or castration-only therapy.

Assessing Radiographic Response to Enzalutamide or Abiraterone post-BAT:

Patients with radiographic progression on CT scan (per RECIST criteria) will stop enzalutamide or abiraterone acetate regardless of PSA response and will come off study. Patients on first line castration-only arm (Cohort C) with radiographic progression will remain on castration therapy but will come off study

Radiographic progression on bone scan will be defined by PCWG2 criteria as ≥ 2 new bone lesions. However, for the first reassessment scan only after treatment with abiraterone or enzalutamide or castration-only therapy, patients with declining PSA should remain on study and have a confirmatory scan performed 12 weeks (3 cycles) later (Appendix 3). If this confirmatory scan shows 2 or more additional new lesions, this defines progression. The date of radiographic progression is the date of the first reassessment bone scan. If the confirmatory scan does not show any additional new lesions, patient remains on study. If progression is observed on subsequent bone scans, a confirmatory scan is not required; the date of this bone scan is the date of progression. Patients with bone scan progression based on these criteria will stop enzalutamide or abiraterone acetate regardless of PSA response and will come off study. Patients on first line castration-only arm (Cohort C) with bone scan progression will remain on castration therapy but will come off study

6.4 Bone Scan with SPECT CT

Patients will have bone scan in addition to SPECT CT obtained at baseline, after 3 months therapy with BAT and after 3 months therapy with either Enzalutamide or

Abiraterone acetate. The Bone scan with SPECT CT scan includes a conventional

planar whole body bone scan coupled with Single-photon emission computed tomography (SPECT) imaging that will allow for quantification of uptake of Tc-99m MDP tracer into areas of bone metastases. This Bone scan with SPECT CT is added at these time points to quantify the effects of BAT or Enzalutamide or Abiraterone acetate on uptake of Tc-99m MDP in an attempt to better understand tumor flare in areas of bony metastases. At all other imaging time points (i.e. every subsequent 3 cycles in in patients responding to BAT or to Enzalutamide or Abiraterone acetate) conventional planar whole body bone scan (i.e. standard bone scan) will be performed. Planar bone scans are compared at each time point to assess response/progression. SPECT CT will only be used to quantify bone uptake of tracer and will not be used to determine radiographic response/progression. Patients on Cohort C will not be offered SPECT CT.

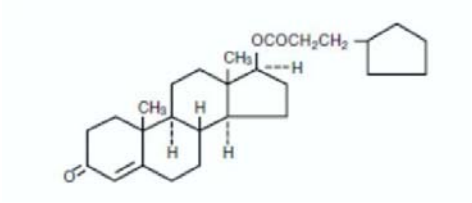
6.5 Safety Assessment

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

7 PHARMACEUTICAL INFORMATION-Testosterone Cypionate and Enanthate

7.1 Testosterone Cypionate Drug Characterization

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



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

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

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

7.1.5 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:

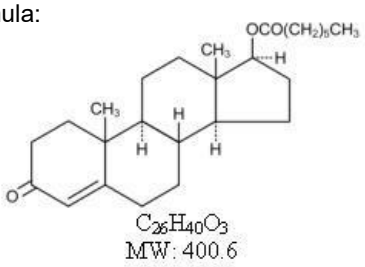
Testosterone cypionate..... 100 mg
Benzyl benzoate ..... 0.1 mL
Cottonseed oil ..... 736 mg
Benzyl alcohol (as preservative) ..... 9.45 mg

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

7.2 Testosterone Enanthate Drug Characterization

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



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

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

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

7.2.5 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.

7.3 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 (38).

7.4 Precautions

7.4.1 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.

7.4.2 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.

7.4.3 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.

7.4.4 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.

7.4.5 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.

7.5 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 hepatitis.
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.

7.5.1 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.

7.5.2 Overdosage

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

7.6 Administration, Supply and Storage

7.6.1 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.

7.6.2 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

7.5.3 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 re-dissolve any crystals that may have formed during storage at low temperatures.

8. STUDY CALENDAR

		BAT Treatment Period (28 day cycles)				Enza/Abi/Castration-Only Treatment Period (28 day cycles)			
	Screening	B1[1] (Day 1)	B2 (Day 29)	B3 (Day 57)	Response Assessment[1,2,3] (Day 85)	PB1[1] (Day 1)	PB2 (Day 29)	PB3 (Day 57)	Response Assessment[1,4,5,6] (Day 85)
History and Physical	X								
Clinic Visit	X	X	X	X	X	X	X	X	X
ECOG PS	X	X	X	X	X	X	X	X	X
CBC	X		X		X	X			X
COMP PANEL	X		X		X	X			X
PSA	X		X	X	X	X	X	X	X
TESTOSTERONE	X		X		X	X			X
LIPID PANEL	X				X				X
ANDROGEN PANEL[7]	X				X				X
METABOLIC PANEL[8]	X				X				X
INFLAMMATION PANEL[9]	X				X				X
PT/PTT	X				X				X
ECG	X								
BONE SCAN with SPECT CT[10]	X				X				X
Standard BONE SCAN[11,14]					BAT Responder every 3 months				Enza or Abi or Castration-only responders every 3 months
CT SCAN[11,14]	X				X				X
Testosterone injection		X	X	X					
Enzalutamide or Abiraterone						X	X	X	
AR CTC SAMPLE[12]	X				X				X
FACIT-F[13]	X				X				X
RANDSF-36	X				X				X
IIEF SURVEY	X				X				X
I-PANAS-SF	X				X				X

BRIEF PAIN INVENTORY	X				X				X

[1]B= BAT Cycle number; PB= Post-BAT cycle number; Enza= Enzalutamide; Abi= Abiraterone

[2]On day 85 of the BAT Treatment Period, patients will be assessed for PSA response. Patients with PSA response will continue to receive T injection every 28 days and the BAT Treatment Period schedule will be repeated until time of PSA progression.

[3]If patient has progression on day 85 of the BAT Treatment Period, they will not receive any therapy and will return in 28 days to start either Enzalutamide or Abiraterone Acetate + Prednisone or Castration-only therapy.

[4]On day 85 of the Enza/Abi/Castration-only Treatment Period, patients will be assessed for PSA response. Patients with PSA response will continue to receive the assigned treatment every 28 days and the Enza/Abi/Castration-only Treatment Period schedule will be repeated until time of PSA progression.

[6]Patients with PSA progression on day 85 of the Enza/Abi/Castration-only Treatment Period will be removed from study.

[7]Androgen panel [dihydrotestosterone (DHT), free testosterone, estradiol, dehydroepiandostenedione sulfate (DHEA-S), DHEA and sex hormone binding globulin (SHBG)] will be done every 3 months x 3 total while on BAT and every 3 months x 2 total while on Enzalutamide or Abiraterone acetate or Castration-only treatment.

[8]Metabolic Panel (fasting glucose, hemoglobin A1c, fasting insulin, serum C-telopeptide, osteocalcin) will be done every 3 months x 3 total while on BAT and every 3 months x 2 total while on Enzalutamide or Abiraterone acetate or Castration-only treatment.

[9]Inflammation Panel (C Reactive Protein) will be done every 3 months x 3 total while on BAT and every 3 months x 2 total while on Enzalutamide or Abiraterone acetate or Castration-only treatment.

[10]Bone scan with SPECT CT is performed at baseline, after 3 cycles of BAT and after 3 cycles of either Enza or Abi. In BAT responders, standard bone scan is performed every subsequent 3 cycles. In Enza or Abi responders, standard bone scan is performed every 3 cycles.

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

[12] AR CTC sample will be collected on screening, Cycle 1 of BAT day 85 and then again post-BAT after 3 cycles on Enza or Abi only (PB Cycle 1 Day 85)

[13]QOL surveys will be done every 3 months x 3 total while on BAT and every 3 months x 2 total while on Enzalutamide or Abiraterone acetate or Castration-only treatment.

[14]For patients who respond to BAT or Enza/Abi/Castration-only therapy for >12 months, it is left to treating physician's discretion to increase CT and bone scan interval to every 4-6 months.

9. DATA MONITORING AND REPORTING REQUIREMENTS

Data and safety monitoring will follow Level I under the SKCCC Data and Safety Monitoring Plan. A copy of this document is available at: http://cro.onc.jhmi.edu/researchCompliance/compliancePlan.pdf

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.

9.1 Adverse Event Monitoring and Reporting

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

9.2 Evaluating Adverse Events

The grade and severity of the event will be determined using the DCT/NCI Common Terminology Criteria, CTCAE v.4.03. A copy of the CTCAE version 4.03can be downloaded from the CTEP home page (http://ctep.cancer.gov). All appropriate treatment areas should have access to a copy of the CTCAE version 4.03. Study staff must use one of the CTCAE criteria to define the event. Adverse events not included in the CTCAE v.4.03 should be reported and graded under the “Other” adverse event within the appropriate category and grade 1 to 5 according to the general grade definitions, mild, moderate, severe, life-threatening, fatal or disabling, as provided in the CTCAE.

The event will be determined to be expected or unexpected.
The determination of whether an AE is expected is based on agent-specific adverse event information provided in Section 7and 8 Pharmaceutical Information. Unexpected AEs are those not listed in the agent-specific adverse event information provided in Section 7and 8 Pharmaceutical Information.

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

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

Based on this information, a decision will be made whether an adverse event should be reported as an expedited report (Serious Adverse Event, section 9.3) 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.

9.2.1 Documenting Adverse Events

Each individual sign or symptom must be documented separately. Worksheets 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.

9.3 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.

9.4 Expedited Reporting

JHM IRB reporting
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://irb.jhmi.edu/Policies/103_6b.html. All deaths on study regardless of attribution must be reported to the JHM IRB.

9.5 Protocol Amendments

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

9.6 Informed consent

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

10. STATISTICAL METHODS

Study design and sample size determination: The joint primary objectives of the trial are to determine the PSA response to enzalutamide or abiraterone acetate or castration-only therapy post-BAT and the PSA response to BAT in men with metastatic CRPC who have progressed on enzalutamide or abiraterone acetate or castration-only therapy without prior treatment of chemotherapy. The PSA response to BAT is defined as ≥50% decrease in serum PSA below the baseline level while on BAT, and PSA response to enzalutamide or abiraterone acetate or castration-only therapy as ≥50% decrease in PSA below nadir level post-BAT. The study will enroll three cohorts of patients: men with metastatic CRPC who have progressed on enzalutamide (Cohort A); men with metastatic CRPC who have progressed on abiraterone acetate (Cohort B); and men with metastatic CRPC who have progressed on first line castration-only therapy (Cohort C). Each cohort will be evaluated separately.

We wish to determine whether the rate of PSA response to re-treatment with enzalutamide or abiraterone acetate or castration-only therapy or the response to BAT

exceeds 5%. The regimen will be considered active in regard to its ability to re-sensitize patients to enzalutamide or abiraterone acetate or castration-only therapy if it results in a ≥25% PSA response rate to enzalutamide or abiraterone acetate or castration-only therapy (i.e. an absolute 20% improvement compared to 5% response rate), and will be considered active in patients who have progressed on enzalutamide or abiraterone acetate or castration-only therapy if it results in a ≥20% PSA response rate to BAT (i.e. an absolute15% increase compared to 5% response rate). The overall type I error will be 0.1, allocated between the two primary endpoints of PSA response to enzalutamide or abiraterone acetate or castration-only therapy (0.05) and PSA response to BAT (0.05).

The trial will enroll up to 90 patients, 30 for each cohort. Thirty evaluable patients will provide 90% power to reject the null 5% PSA response to enzalutamide/abiraterone acetate/castration-only therapy in favor of 25% PSA response with a one-sided test at significance level 0.05. For the co-primary endpoint of PSA response to BAT, a total of 30 patients will provide 83% power to reject the null 5% PSA response to BAT in favor of 20% response to BAT. The patients who do not receive at least 3 cycles of BAT will be replaced.

One interim analysis and 1 final analysis are planned for PSA response to re-treatment with enzalutamide or abiraterone acetate or castration-only and 1 final analysis is planned for PSA response to BAT. An interim analysis will be conducted to assess futility after PSA response data to enzalutamide or abiraterone acetate or castration-only therapy is available for the first 9 patients. According to the optimal Simon's two-stage design, if none of the patients respond to re-treatment with enzalutamide or abiraterone acetate or castration-only therapy, the re-treatment portion of the study will be terminated. At that point, enrollment will only continue to the portion of the study evaluating BAT therapy. If there is at least 1 response upon re-treatment with enzalutamide or abiraterone acetate or castration-only therapy, the re-treatment portion of the study will continue and an additional 21 patients will be re-treated with enzalutamide or abiraterone acetate or castration-only therapy. If a total of 4 or more

subjects have a PSA response to enzalutamide or abiraterone acetate or castration-only therapy, we will conclude that this regimen is promising as a means to re-sensitize to enzalutamide or abiraterone acetate or castration-only therapy. If 4 or more PSA responses to BAT are observed out of the 30 patients enrolled, we will conclude that BAT is promising in patients who have progressed on enzalutamide or abiraterone acetate or castration-only therapy.

The evaluable population includes the subjects who complete at least 3 cycles of BAT. The primary analysis will be performed in the evaluable population. The analysis including all patients who receive at least one dose of enzalutamide or abiraterone acetate will serve as supportive.

Analysis methods for efficacy endpoints: The co-primary endpoints will be the overall PSA response rate to BAT and the overall PSA response rate to enzalutamide or abiraterone acetate or castration-only therapy post-BAT. We will estimate the PSA response rates as the proportions of patients who have a ≥50% decrease in serum PSA below the baseline level while on BAT and those who have a ≥50% decrease in PSA below nadir level post-BAT with repeated treatment of enzalutamide or abiraterone acetate or castration-only therapy, respectively. Subjects who receive at least 3 cycles of BAT but have unknown or missing PSA response will be treated as non-responders, and therefore be included in the denominator when calculating the PSA response rates. 95% confidence interval will be reported. Additionally, we will assess the percentage of patients who show any PSA decline from the baseline while on BAT. The analysis will be performed separately for the three cohorts, as well as the three cohorts combined.

The first secondary endpoint is the time to PSA progression with BAT. Time to PSA progression on BAT is defined as the time from the date of initial dose of T to the date of the PSA measurement when it shows an ≥25% increase above the nadir value and confirmed by a repeat PSA 4 weeks later (PCWG2 criteria) during the treatment with BAT, and will be described using Kaplan-Meier method.

The second secondary endpoint is the time to PSA progression on enzalutamide or abiraterone acetate or castration-only therapy post-BAT, defined as the time from the date of re-initiation of enzalutamide or abiraterone acetate or castration-only therapy to the date of the PSA measurement when it shows an increase by ≥25% above the nadir value that occurred following re-initiation of enzalutamide or abiraterone acetate or castration-only therapy and confirmed by a repeat PSA 4 weeks later (PCWG2). We will summarize it using Kaplan-Meier method.

The third secondary endpoint is the objective response with BAT and with enzalutamide or abiraterone acetate or castration-only therapy following BAT, respectively, in patients with measurable disease. Tumor response will be evaluated based on RECIST 1.1 for soft tissue lesions and on PCWG2 criteria for bone lesions. Time to radiographic progression with post-BAT enzalutamide or abiraterone acetate or castration-only therapy is defined as the time from the date of initiation of enzalutamide or abiraterone acetate or castration-only therapy post BAT to the date of new lesions on bone scan (per PCWG2 criteria) or progression on CT scan (per RECIST criteria) compared to baseline. We will estimate the response rates, and summarize time to progression using Kaplan-Meier method.

The fourth secondary endpoint is the time to initiation of docetaxel chemotherapy, defined as the time from the start date of BAT to the date of initial dose of docetaxel, and will be described using Kaplan-Meier method.

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 and pain will be assessed using IIEF and BPI, respectively. For each module, summary statistics of the scores will be reported at baseline, after 3 cycles of BAT x 3 total and after 3 cycles x 2 total of either enzalutamide or abiraterone acetate or castration-only therapy. In each cohort changes in quality of life scores pre- and post-treatment will be evaluated at each follow-up time by paired-sample t-tests or Wilcoxon signed rank tests as appropriate. In addition, mixture effect models will be fitted for accessing the quality of

life changes over time. The change of metabolic measures (e.g., lipid profile, metabolic panel, androgen panel, inflammation panel, BMI) will be evaluated using the same analysis methods.

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

11. References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics, 2008. CA Cancer J Clin. 2008;58:71-96.
2. Huggins C, Hodges CV (1941) Studies on prostatic cancer. I. The effect of castration, of estrogen and androgen injection on serum phosphatases in metastatic carcinoma of the prostate. Cancer Res 1(4): 293-297
3. Crawford ED, Eisenberger MA, McLeod DG, Spaulding JT, Benson R, Dorr FA, Blumenstein BA, Davis MA, Goodman PJ.A controlled trial of leuprolide with and without flutamide in prostatic carcinoma. N Engl J Med. 1989;321:419-24.
4. Laufer M, Denmeade SR, Sinibaldi VJ, Carducci MA, Eisenberger MA.Complete androgen blockade for prostate cancer: what went wrong? J Urol. 2000;164:3-9.
5. Linja MJ, Savinainen KJ, Saramaki OR, Tammela TL, Vessella RL & Visakorpi T 2001 Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. Cancer Res 61 3550-3555.
6. Shah RB, Mehra R, Chinnaiyan AM, Shen R, Ghosh D, Zhou M, Macvicar GR, Varambally S, Harwood J, Bismar TA, et al. Androgen-independent prostate cancer is a heterogeneous group of diseases: lessons from a rapid autopsy program. Cancer Res 2004;64:9209-9216.
7. de Bono JS, Logothetis CJ, Molina A, Fizaki K, North S, Chu L, Chi KN, Jones RJ, Goodman OB, Saad F, Staffurth JN, Mainwaring P, Harland S, Flaig TW, Hutson TE, Cheng T, Patterson H, Hainsworth JD, Ryan CJ, Sternberg CN, Ellard SL, Flechon A, Saleh M, Scholz M, Efstathiou E, Zivi A, Bianchini D, Loriot Y, Chieffo N, Kheoh T, Haqq CM, Scher HI, Cou-AA-301 Investigators. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med 2011;364:1995-2005. PMCID:PMC3471149
8. Scher HI, Fizazi K, Saad F, Taplin ME, Sternberg CN, Miller K, de Wit R, Mulders P, Chi KN, Shore ND, Armstrong AJ, Flaig TW, Fléchon A, Mainwaring P, Fleming M, Hainsworth JD, Hirmand M, Selby B, Seely L, de Bono JS; AFFIRM Investigators. Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med. 2012;367:1187-97.
9. Loriot Y, et al. Antitumour activity of abiraterone acetate against metastatic castration-resistant prostate cancer progressing after docetaxel and enzalutamide (MDV3100). Ann Oncol 2013;24:1807-12.
10. Noonan KL, North S, Bitting RL, Armstrong AJ, Ellard SL, Chi KN. Clinical activity of abiraterone acetate in patients with metastatic castration-resistant prostate cancer progressing after enzalutamide. Ann Oncol 2013;24:1802-7.
11. Schrader AJ, Boegemann M, Ohlmann CH, Schnoeller TJ, Krabbe LM, Hajili T, Jentzmik F, Stoeckle M, Schrader M, Herrmann E, Cronauer MV. Enzalutamide in Castration-resistant Prostate Cancer Patients Progressing After Docetaxel and Abiraterone. Eur Urol. 2013 Jul 2. [Epub ahead of print]

12. Bianchini D, Lorente D, Rodriguez-Vida A, Omlin A, Pezaro C, Ferraldeschi R, Zivi A, Attard G, Chowdhury S, de Bono JS. Antitumour activity of enzalutamide (MDV3100) in patients with metastatic castration-resistant prostate cancer (CRPC) pre-treated with docetaxel and abiraterone. Eur J Cancer. 2013 Sep 25. [Epub ahead of print]
13. Chen CD, Welsbie DS, Tran C, Baek SH, Vessella R, Rosenfeld MG, Sawyers CL. Molecular determinants of resistance to antiandrogen therapy. Nat Med 2004;10:33-9.
14. Isaacs JT, D'Antonio JM, Chen S, Antony L, Dalrymple SP, Ndikuyeze GH, Luo J, Denmeade SR. Adaptive auto-regulation of androgen receptor provides a paradigm shifting rationale for bipolar androgen therapy (BAT) for castrate resistant human prostate cancer. Prostate. 2012;72:1491-505. PMCID:PMC3374010
15. Dehm SM, Schmidt LJ, Heemers HV, Vessella RL, Tindall DJ. Splicing of a novel androgen receptor exon generates a constitutively active androgen receptor that mediates prostate cancer therapy resistance. Cancer Res. 2008;68:5469-77. PMCID:PMC2663383
16. Li Y, Chan SC, Brand LJ, Hwang TH, Silverstein KA, Dehm SM. Androgen receptor splice variants mediate enzalutamide resistance in castration-resistant prostate cancer cell lines. Cancer Res. 2013;73:483-9. PMCID:PMC3549016
17. Hu R, Dunn TA, Wei S, Isharwal S, Veltri RW, Humphreys E, Han M, Partin AW, Vessella RL, Isaacs WB, Bova GS, Luo J. Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. Cancer Res. 2009;69:16-22. PMCID: PMC2614301.
18. Sun S, Sprenger CC, Vessella RL, Haugk K, Soriano K, Mostaghel EA, Page ST, Coleman IM, Nguyen HM, Sun H, Nelson PS, Plymate SR. Castration resistance in human prostate cancer is conferred by a frequently occurring androgen receptor splice variant. J Clin Invest. 2010;120:2715-30. PMCID:PMC2912187
19. Linja MJ, Savinainen KJ, Saramaki OR, Tammela TL, Vessella RL, Visakorpi T. Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. Cancer Res 2001;61:3550-55.
20. Vander Griend DJ & Isaacs JT 2008 Androgen Receptor as a Licensing Factor for DNA Replication. In Androgen Action in Prostate Cancer. Eds DJ Tindall & JL Mohler: Springer, In Press.
21. Langeler EG, van Uffelen CJ, Blankenstein MA, van Steenbrugge GJ & Mulder E. Effect of culture conditions on androgen sensitivity of the human prostatic cancer cell line LNCaP. Prostate 1993;23:213-223.
22. Kokontis JM, Hay N, Liao S. Progression of LNCaP prostate tumor cells during androgen deprivation: hormone-independent growth, repression of proliferation by androgen, and role for p27Kip1 in androgen-induced cell cycle arrest. Mol Endocrinol 1998;12:941-953.
23. Kokontis J, Takakura K, Hay N, Liao S. Increased androgen receptor activity and altered c-myc expression in prostate cancer cells after long-term androgen deprivation Cancer Res. 1994;54:1566-73.

24. Umekita Y, Hiipakka RA, Kokontis JM, Liao S. Human prostate tumor growth in athymic mice: inhibition by androgens and stimulation by finasteride. Proc Natl Acad Sci U S A. 1996;93:11802-7.
25. Chuu CP, Hiipakka RA, Fukuchi J, Kokontis JM, Liao S. Androgen causes growth suppression and reversion of androgen-independent prostate cancer xenografts to an androgen-stimulated phenotype in athymic mice. Cancer Res. 2005 Mar 15;65:2082-4.
26. Litvinov IV, Vander Griend DJ, Antony L, Dalrymple S, De Marzo AM, Drake CG, Isaacs JT. Androgen receptor as a licensing factor for DNA replication in androgen-sensitive prostate cancer cells. Proc Natl Acad Sci U S A 2006;103: 15085-15090.
27. Vander Griend DJ, Litvinov IV, Isaacs JT. Stabilizing androgen receptor in mitosis inhibits prostate cancer proliferation. Cell Cycle 2007;6: 647-651
28. Haffner, MC, Aryree MJ, Toubaji A, Esopi DM, Albadine R, Gurel B, Isaacs WB, Bova GS, Liu W, Xu J, Meeker AK, Netto G, De Marzo AM, Nelson WG, Yegnasubramanian S. Androgen -induced TOP2B-mediated double-strand breaks and prostate cancer gene rearrangements. Nat Genet 2010;42:668-75. PMCID:PMC3157086
29. Hu R, Lu C, Mostaghel EA, Yegnasubramanian S, Gurel M, Tannahill C, Edwards J, Isaacs W, Nelson PS, Bluemn E, Plymate SR, Luo J. Distinct transcriptional programs mediated by the ligand-dependent full-length androgen receptor and its splice variants in castration-resistant prostate cancer. Cancer Res. 2012;72:3457-62. PMCID:PMC3415705
30. Thelen P, Heinrich E, Bremmer F, Trojan L, Strauss A. Testosterone boosts for treatment of castration resistant prostate cancer: An experimental implementation of intermittent androgen deprivation. Prostate 2013;73:1699-709.
31. Denmeade SR, Isaacs JT. Bipolar androgen therapy: the rationale for rapid cycling of supraphysiologic androgen/ablation in men with castration resistant prostate cancer. Prostate. 2010;70:1600-7.
32. Cai C, Chen S, Ng P, Bubley GJ, Nelson PS, Mostaghel EA, Marck B, Matsumoto AM, Simon NI, Wang H, Chen S, Balk SP. Intratumoral de novo steroid synthesis activates androgen receptor in castration-resistant prostate cancer and is upregulated by treatment with CYP17A1 inhibitors. Cancer Res. 2011 Oct 15;71(20):6503-13.
33. Brendler H, Chase WE, Scott WW. Prostatic Cancer: Further investigation of hormone relationships. Arch Surg 1949;433-440.
34. Prout GR, Brewer WR. Response of men with advanced prostatic carcinoma to exogenous administration of testosterone. Cancer 1967;20:1871-1878.
35. Szmulewitz R, Mohile S, Posadas E, Kunnavakkam R, Karrison T, Manchen E, Stadler WM. A Randomized Phase 1 Study of Testosterone Replacement for Patients with Low-Risk Castration-Resistant Prostate Cancer. Eur Urol. 2009 Feb 27. [Epub ahead of print]

36. Morris MJ, Huang D, Kelly WK, Slovin SF, Stephenson RD, Eicher C, Delacruz A, Curley T, Schwartz LH, Scher HI. Phase 1 trial of high-dose exogenous testosterone in patients with castration-resistant metastatic prostate cancer. Eur Urol. 2009 Aug;56(2):237-44. Epub 2009 Apr 3.
37. Hussain MH, Pienta KJ, Redman BG, Cummings GD, Flaherty LE. Oral etoposide in the treatment of hormone-refractory prostate cancer. Cancer. 1994;74:100-3.
38. Behre HM, Nieschlag E.Testosterone preparations for clinical use in males. In: Testosterone: Action, Deficiency, Substitution 4th Edition (E Nieschlag, H.M. Behre, eds.) Cambridge University Press, Cambridge UK, 2012, pp.309-35.
39. Simon R. Optimal two-stage designs for phase II clinical trials. Contr Clin Trials 1989; 10:1-10.

Appendix I

Response Evaluation Criteria in Solid Tumors (RECIST) Quick Reference

Eligibility

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

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

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

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

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

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

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

Methods of Measurement

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

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

When the primary endpoint of the study is objective response evaluation, ultrasound (US) should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions

and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

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

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

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

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

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

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

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

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

Response Criteria

Evaluation of Target Lesions

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

Evaluation of Non-Target Lesions

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

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

Evaluation of Best Overall Response

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

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

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

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

Confirmation

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

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

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

Duration of Overall Response

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

Duration of Stable Disease

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

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

Response Review

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

Reporting of Results

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

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

All conclusions should be based on all eligible patients.

Sub-analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.).

However, these sub-analyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported.

The 95% confidence intervals should be provided.

Appendix II
NCI COMMON TOXICITY CRITERIA, VERSION 4.03

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

Appendix 3: Bone Scan Assessment