HHS Public Access

Author manuscript

Sci Transl Med. Author manuscript; available in PMC 2015 July 20.

Published in final edited form as:

Sci Transl Med. 2015 January 7; 7(269): 269ra2. doi:10.1126/scitranslmed.3010563.

Effect of bipolar androgen therapy for asymptomatic men with castration-resistant prostate cancer: Results from a pilot clinical study

Michael T. Schweizer[*,†], **Emmanuel S. Antonarakis**, **Hao Wang**, **A. Seun Ajiboye**, **Avery Spitz**, **Haiyi Cao**, **Jun Luo**, **Michael C. Haffner**, **Srinivasan Yegnasubramanian**, **Michael A. Carducci**, **Mario A. Eisenberger**, **John T. Isaacs**, and **Samuel R. Denmeade**[†]

The Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA

Abstract

Targeting androgen receptor (AR) axis signaling by disrupting androgen-AR interactions remains the primary treatment for metastatic prostate cancer. Unfortunately, all men develop resistance to primary castrating therapy and secondary androgen deprivation therapies (ADTs). Resistance develops in part because castration-resistant prostate cancer (CRPC) cells adaptively up-regulate AR levels through overexpression, amplification, and expression of ligand-independent variants in response to chronic exposure to a low-testosterone environment. However, preclinical models suggest that AR overexpression represents a therapeutic liability that can be exploited via exposure to supraphysiologic testosterone to promote CRPC cell death. Preclinical data supported a pilot study in which 16 asymptomatic CRPC patients with low to moderate metastatic burden were treated with testosterone cypionate (400 mg intramuscular; day 1 of 28) and etoposide (100 mg oral daily; days 1 to 14 of 28). After three cycles, those with a declining prostate-specific antigen (PSA) continued on intermittent testosterone therapy monotherapy. Castrating therapy was continued to suppress endogenous testosterone production, allowing for rapid cycling from supraphysiologic to near-castrate serum testosterone levels, a strategy termed bipolar androgen therapy (BAT). BAT was well tolerated and resulted in high rates of PSA (7 of 14 evaluable patients) and radiographic responses (5 of 10 evaluable patients). Although all men showed eventual PSA progression, four men remained on BAT for ≥1 year. All patients (10 of 10) demonstrated PSA reductions upon receiving androgen-ablative therapies after BAT, suggesting that BAT may also restore sensitivity to ADTs. BAT shows promise as treatment for CRPC and should be further evaluated in larger trials.

[†]Corresponding author. denmesa@jhmi.edu (S.R.D.); schweize@uw.edu (M.T.S.).

[*]Present address: Division of Oncology, Department of Medicine, University of Washington/Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA.

Author contributions: S.R.D., J.T.I., M.T.S., E.S.A., and H.W. designed the research; S.R.D., M.T.S., E.S.A., A.S., H.C., M.A.C., M.A.E., A.S.A., J.L., M.C.H., S.Y., and J.T.I. performed the research; H.W. contributed analytic tools; S.R.D., H.W., and M.T.S. analyzed the data; M.T.S. and S.R.D. wrote the paper.

Competing interests: S.R.D. is a consultant for Sophiris, GenSpera, and Medicenna. The other authors declare that they have no competing interests.

Introduction

The discovery by Dr. Charles Huggins in 1941 of the remarkable palliative benefit of androgen deprivation therapy (ADT) via surgical castration or estrogen therapy in men with symptomatic, advanced prostate cancer was a transformative event in the history of medicine (1,2). Over the ensuing 70 years, ADT has remained the mainstay of treatment and is now used routinely in men with asymptomatic disease, despite side effects that include impotence, hot flashes, fatigue, and decreased functional activity. In addition, men suffer from sequelae of the castration-induced metabolic syndrome, such as loss of lean muscle and bone mass, anemia, and weight gain (3–6). From the outset of ADT use, it was recognized that all men eventually develop castration-resistant prostate cancer (CRPC) that was presumed to be due to sustained androgen receptor (AR) signaling via a number of different mechanisms (7–13). Thus, the prevailing treatment paradigm has been to block ligand-dependent AR activity by any means necessary. This hypothesis led to the development of “second-line” therapies aimed at further blocking androgen ligand signaling through AR The culmination of this reasoning has been the development of the CYP17 inhibitor abiraterone acetate, an androgen synthesis inhibitor, and enzalutamide, a potent antiandrogen. Both agents received U.S. Food and Drug Administration (FDA) approval based on modest survival benefit compared to placebo in men with metastatic CRPC (14–16).

Unfortunately, resistance to these agents develops quickly. Additionally, emerging evidence suggests a reduced response when abiraterone acetate and enzalutamide are used as “third-line” therapy (17–20). Resistance first manifests as a sustained rise in the androgen-responsive gene PSA (prostate-specific antigen), consistent with reactivation of a functioning AR axis. Evaluation of clinical material demonstrates that CRPC cells remain addicted to AR signaling and adapt to chronic exposure to androgen-ablative therapies through an autoregulatory increase in AR activity by a variety of mechanisms that include increased expression of wild-type AR and ligand-independent AR variants, *AR* gene amplification, and *AR* mutations (7–13,21). Data from a variety of sources, including rapid autopsy programs, have demonstrated that AR expression persists even in men with CRPC who have died from prostate cancer after chronic ADT and multiple types of second-line hormonal therapies (22–24). Chen *et al.* demonstrated that prostate cancer cell lines adapt to serial passage in castrated mice through an autoregulatory increase in AR expression that is sufficient to induce resistance to both ADT and the antiandrogen bicalutamide (25). Studies in our own laboratory have documented that AR levels increased 30- to 90-fold in CRPC cell lines compared to normal prostate cells (26). Using clinical samples, we further demonstrated a marked AR increase in men progressing from castration-sensitive cancer to a CRPC state (26). In addition to up-regulation of the full-length AR (AR-FL), human AR-expressing prostate cancer cells can up-regulate expression of truncated ligand-independent AR variants upon exposure to androgen-ablative therapies. Expression of these ligand-independent variants is associated with resistance in preclinical models and clinical studies (27–32).

Against this background of renewed interest in more potent blockade of ligand-dependent AR signaling, there has been the long-standing observation that the growth of AR-positive

human CRPC cell lines can be inhibited by supraphysiologic levels of androgens (26,33). Several complementary mechanisms for this paradoxical effect have been described. Isaacs *et al.* demonstrated that AR is a DNA licensing factor that plays a critical role in DNA replication and must be degraded as a cell goes through the cell cycle (26, 34–36). In the presence of supraphysiologic testosterone, increased ligand-bound AR in the nucleus is stabilized against degradation. Lack of AR degradation due to overstabilization inhibits DNA relicensing, resulting in cell death in the subsequent cycle (35). Haffner *et al.* showed that dihydrotestosterone (DHT) generates transient double-strand DNA breaks (DSBs) in CRPC cells through the recruitment of AR and topoisomerase IIβ (TOP2B) to androgen response elements (AREs) (37, 38). Although single-agent etoposide had minimal activity in a previous study in men with CRPC, it may have the ability to potentiate DSBs induced by testosterone through its ability to inhibit TOP2B and DNA repair (39). Various studies have also shown that resistance to ADT, abiraterone acetate, and enzalutamide may be mediated through increased expression of AR splice variants that remain transcriptionally active despite loss of the AR ligand-binding domain (27–32).

These preclinical studies suggested that the adaptive overexpression of AR by CRPC cells could potentially be targeted through the administration of sufficient systemic testosterone to achieve supraphysiologic serum testosterone levels. We refer to this approach as bipolar androgen therapy (BAT) (40). The term “bipolar” is used to emphasize that, with this strategy, there is rapid cycling between two polar extremes: from supraphysiologic serum testosterone levels achieved through intramuscular injection of 400 mg of testosterone cypionate back to near-castrate testosterone levels over a 4-week cycle. CRPC cells expressing high AR levels would be vulnerable to cell death when exposed to supraphysiologic testosterone because of inability to completely degrade high levels of androgen-stabilized nuclear AR Supraphysiologic testosterone may also induce lethal DSBs in prostate cancer cells chronically starved of androgen. Finally, because of the bipolar nature of the therapy, CRPC cells that survive high testosterone due to baseline low AR levels or through adaptive down-regulation of AR would become vulnerable to death when suddenly re-exposed to low testosterone over the course of a treatment cycle.

On the basis of the combined results demonstrating the effects of supraphysiologic androgen on prevention of DNA relicensing and the potential for production of stabilized DSBs in conjunction with etoposide, we conducted a single-site, single-arm pilot study in men with CRPC who progressed on ADT and second-line androgen-ablative therapies to evaluate the safety and efficacy of BAT in combination with oral etoposide.

Results

Androgen-induced inhibition of growth of AR-overexpressing CRPC cells

The in vitro growth of some AR-expressing human prostate cancer cell lines, particularly those that have been adapted to grow in media lacking androgens (that is, charcoal-stripped serum-containing media), can be markedly inhibited by exposure to supraphysiologic levels of androgen in the media. This inhibition is observed by exposure to 10 nM R1881, a synthetic androgen that is non-aromatizable and is poorly bound by steroid hormone binding globulin (Fig. 1A). This concentration of R1881 is considered a supraphysiologic amount of

androgen because it is ∼20-fold higher than the free testosterone concentration in the serum of intact adult human males and ∼1000-fold higher than the free testosterone level in 10% fetal bovine serum– containing culture media (41).

These adapted cell lines are typically highly resistant to growth inhibition by antiandrogens such as bicalutamide (Fig. 1A). Growth inhibition by androgens is dose-dependent and is only observed in a subset of AR-positive human prostate cancer cell lines (Fig. 1B). Previous studies exploring the mechanisms underlying this paradoxical growth inhibition have found that androgen exposure can induce DSBs and induce G_1 arrest through reduction in expression of S-phase kinase-associated protein 2 (Skp2) and c-Myc, and induction of p27(Kip1) (37, 38, 42). Exposure of LNCaP cells to DHT induces DNA breaks as measured by phosphorylation of H2A histone family, member X to a form known as γH2A.x as a reaction to DSBs (Fig. 1C). These γH2A.x foci indicative of DSBs, as well as other markers of DSBs (such as binding of biotin-conjugated nucleotides, ATM recruitment, or dual fluorescence in situ hybridization probe hybridization), are transient because they appear within a few hours of exposure and are no longer detectable after 24 hours (37). Hormone-induced DSBs are likely generated by TOP2B, a class 2 topoisomerase that has been shown to induce DSBs to relieve topological constraints (37). An additive effect on DSBs occurs when androgen stimulation is combined with the topoisomerase 2 poison etoposide (Fig. 1C), which covalently traps catalytically active TOP2 on DNA, resulting in stabilization of DSBs.

Recently, Isaacs *et al.* demonstrated that AR is involved in the process of DNA licensing, in which a complex of proteins known as licensing factors assemble at origins of replication to form origin of replication complexes required to begin DNA replication (26, 34–36). Once the cell completes the cell cycle, relicensing must occur for a subsequent round of replication. For relicensing to occur, replication complexes (RCs) must be removed from origin of replication sites (ORSs) during G_2-mitosis, so that in early G_1 of the next cell cycle, ORSs are fully accessible to bind newly synthesized RC proteins and initiate formation of new pre-RCs. Evaluation of AR levels during cell cycle progression of castration-resistant LNCaP cells growing in a castrate host documented that AR is degraded via the proteasome during mitosis and rapidly resynthesized in early G_1 (34). When grown under castrate conditions, high AR–expressing LNCaP cells continued to proliferate. In contrast, when these castration-resistant LNCaP xenografts were treated with testosterone implants to achieve supraphysiologic serum testosterone levels, marked growth inhibition was observed (Fig. 1, D and E). These growth-inhibited xenografts had similar amount of cells with nuclear AR, Ki-67 positivity, and mitotic index compared to xenografts growing in castrate animals (Fig. 1E). However, about threefold increase in the cell death index demonstrates that growth inhibition was not due to a cytostatic effect of supraphysiologic testosterone but rather to induction of cell death in treated xenografts. More strikingly, the percentage of cells staining positive for AR in mitosis was about 11-fold higher in cells exposed to supraphysiologic testosterone versus castrate-only animals (Fig. 1, E and F). Although testosterone treatment produced growth inhibition, levels of PSA/gram of tumor were about fourfold higher in these testosterone-inhibited LNCaP cells, demonstrating that the AR axis is still functional (Fig. 1E).

Clinical study of BAT and etoposide in men with CRPC

On the basis of these preclinical results, we designed an open-label, single-site, single-arm pilot study in men with CRPC to evaluate the safety and efficacy of BAT in combination with etoposide (Fig. 2A). All subjects were maintained on ADT with a luteinizing hormone-releasing hormone (LHRH) agonist (for example, goserelin, leuprolide, and triptorelin) to suppress endogenous testicular androgen synthesis, thus allowing for rapid testosterone cycling. In the initial stage of the treatment, subjects received three 28-day cycles of combination testosterone cypionate and etoposide. On day 1, the patients received a 400-mg intramuscular injection of testosterone cypionate. On days 1 to 14, the patients received oral etoposide, 100 mg daily. This FDA-approved dose of testosterone cypionate was selected on the basis of previous pharmacokinetic studies demonstrating that this dose and formulation produce supraphysiologic testosterone levels (>1500 ng/dl) within the first few days after injection, with a subsequent decline to high-normal testosterone levels after 2 weeks, and a return to near-castrate testosterone levels by 28 days (43). The dose of etoposide was selected on the basis of a previous phase 2 trial in men with CRPC (39). Patients were eligible to continue on BAT alone beyond the first three cycles if they had a PSA decline (PSA below the prestudy baseline) or a PSA that was trending downward after the first three cycles of combination testosterone plus etoposide and was no more than 50% above the prestudy baseline level. The primary endpoint of the study was the rate of PSA decline below baseline after three cycles of BAT plus etoposide. Evaluable patients were those completing at least three cycles of combined therapy.

Between March 2010 and February 2013, a total of 16 subjects were screened and enrolled on this study, which was conducted at the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins. The median follow-up time for those enrolled was 124.5 days (range, 35 to 546 days). Fourteen of 16 patients were evaluable for response, having completed the initial phase of therapy (three cycles of combination testosterone cypionate plus etoposide). Two patients were not evaluable because they came off study after only one cycle of therapy due to toxicity. Seven of 14 (50%) subjects who completed the three initial cycles of combination therapy demonstrated a PSA response. These patients proceeded to the second phase of the study and were treated with BAT only, without further etoposide. The median age at the time of screening was 71 years (range, 56 to 87 years), and median baseline PSA was 21.7 ng/ml (range, 1.4 to 819.1 ng/ml). The median duration of previous androgen deprivation was 45.4 months (range, 12 to 146 months). Other baseline characteristics are summarized in Fig. 2B.

PSA reductions

In most patients tested, the serum testosterone level was supraphysiologic (normal serum testosterone level in men over age 50 years ranges from 130 to 700 ng/dl), with a mean >1500 ng/dl (range, 920 to >3200 ng/dl) at 2 days after testosterone injection (Fig. 2C) (44). Two weeks after testosterone injection, average testosterone levels remained above 600 ng/dl, and by 28 days, the levels averaged ∼150 ng/dl (Fig. 2C). No man returned to castrate (<50 ng/dl) testosterone levels after 28 days across all cycles of therapy. Six of 14 [42.9%; 95% confidence interval (CI), 20.6 to 68.8%] subjects completing the initial stage had a PSA decline after three cycles of testosterone cypionate plus etoposide, and one additional patient

(subject 15) achieved a PSA reduction during the BAT-only stage [*n* = 7 total (50%; 95% CI, 23.0 to 77.0%) in the "responder group"] (Table 1). Thus, the study met the primary PSA endpoint of at least three subjects with a PSA decline below baseline. Four (28.6%) subjects had a PSA decline ≥50% compared to baseline (see column marked "On-study PSA low relative to baseline (%)," Table 1, for values), and two other patients experienced PSA declines of 48 and 47% (Fig. 3A).

Three patterns of PSA change were observed during the first three cycles of BAT plus etoposide (Fig. 3B and fig. S1). In pattern 1 (*n* = 7), patients had an overall increasing PSA level over the three cycles of therapy. This increase was linear or mirrored serum testosterone levels, increasing when testosterone levels were high and dropping as serum testosterone levels dropped over the 28-day cycle. These patients were considered non-responders and removed from the study after three cycles.In pattern 2 (*n* = 3), PSA levels initially increased with the first dose of testosterone cypionate and then steadily declined to below baseline levels. In pattern 3 (*n* = 4), there was no initial spike in PSA; instead, the PSA levels declined immediately by >50% after the first cycle of therapy. Patients exhibiting the latter two patterns were offered the option to continue on BAT alone in the second phase of treatment after the initial three cycles of combination therapy. Figure 3C shows an example of the PSA change in an individual patient (subject 9) during 3 cycles of BAT plus etoposide followed by 13 cycles of BAT alone and then a return to castrate testosterone levels (consistent with pattern 2).

All seven subjects who responded to therapy eventually displayed PSA progression, with a median time to PSA progression of 221 days (range, 95 to 454 days) for this group (Table 1). Despite PSA progression, five of the seven responders were considered to still be deriving benefit from therapy based on lack of radiographic progression and increased subjective quality of life. Four of these men remained on BAT for ≥12 cycles, and one patient remains on study after 22 cycles. The median duration of clinical benefit (defined as time on study) for the responders was 343 days (range, 91 to not reached). Notably, the group of subjects with PSA declines (*n* = 7) had a significantly higher mean baseline PSA compared to those who did not respond (159.7 ng/ml versus 13.9 ng/ml, *P* = 0.019). A graphical summary of the changes in serum PSA for each subject enrolled is presented in fig. S1.

Radiographic responses

At baseline, 10 subjects had Response Evaluation Criteria in Solid Tumors (RECIST)–evaluable soft tissue metastases (Table 1). Of these patients, two (20%) had progressive disease (PD), three (30%) had stable disease after a median follow-up of 91 days (range, 87 to 92 days), four (40%) had PRs, and one (10%) had a CR. One patient (subject 4) with an initial PR developed PD with continued treatment. No other patients with an initial PR or CR developed radiographic progression after a median follow-up of 123.5 days (range, 87 to 501 days). An example of a CR (patient 16) and a PR (patient 15) in lymph node metastases after three cycles of BAT plus etoposide is shown in Fig. 3D. Three subjects had baseline osseous metastases. No one had progressive bone metastatic disease per Prostate Cancer Working Group 2 (PCWG2) criteria; however, one subject (subject 8) was taken off study

because of the development of a single new osseous metastasis not meeting the PCWG2 definition for progression. This lesion was felt to be evidence of clinical progression.

PSA reductions with subsequent hormonal therapies

We performed a post hoc exploratory analysis on the effect of BAT on subsequent hormonal therapies. Overall, 12 of 13 subjects had PSA decline to AR-directed therapy given after BAT [one patient continued to show PSA progression upon return to castrate testosterone levels and proceeded to receive docetaxel (subject 8), and the 14th patient remains on BAT] (Table 2). For 12 patients, testosterone levels were allowed to return to the castrate range after completion of the study, and within 1 month of renewed PSA progression, the patients were placed back on a second-line AR-directed therapy (such as abiraterone, enzalutamide, or bicalutamide). Of these 12 subjects, 9 (75%) had a PSA decline below their end-of-study PSA. Of the six PSA responders who came off study, four (66.7%) had a PSA decline below their end-of-study PSA upon becoming castrate again. All of the patients had received at least one antiandrogen before starting the study (Table 2). Ten of 10 (100%) patients receiving second-line therapy with either abiraterone (n = 4 of 4) or an antiandrogen [enzalutamide (n = 4 of 4), bicalutamide (n = 1 of 1), nilutamide (n = 1 of 1)] had a PSA decline (range, 30.8 to 99.5%). Examples of PSA and objective response for patients 4 and 14 are shown in Fig. 4 (A to C). Four of four patients receiving abiraterone and three of four patients on enzalutamide had >50% PSA decline. Notably, two subjects were rechallenged with a first-generation antiandrogen (nilutamide or bicalutamide) and one with enzalutamide after having previously progressed on these agents. These subjects achieved a 44.3, 30.9, and 53.2% PSA decline upon initiation of nilutamide, bicalutamide, and enzalutamide, respectively. The patient rechallenged with enzalutamide had also previously progressed on abiraterone before enrolling in this study.

Mechanistically, this resensitization effect may be due to the ability of high-dose androgens to acutely down-regulate AR-FL expression and eliminate expression of ligand-independent AR splice variants (Fig. 4D). The expression of these AR splice variants has been associated with resistance to ADT, abiraterone, and enzalutamide (27–32). However, the AR-V7 variant is not expressed in every resistant AR-positive human prostate cancer cell line (Fig. 4E). In addition, some lines, such as LNCaP, lack expression of the AR variants and are highly sensitive to growth inhibition by androgen. In contrast, the CWR22-Rv1 cells express high levels of AR-V7, and the expression of this variant is not down-regulated by exposure to R1881 or up-regulated upon exposure to enzalutamide, as occurs in the AR-V7–positive VCaP line (Fig. 4E).

Adverse events

Most adverse events (AEs) occurred during the initial phase of treatment and were largely consistent with known side effects of etoposide. Initial-phase side effects were mostly low-grade (grade ≤2) and included nausea (n = 10), fatigue (n = 9), alopecia (n = 9), edema (n = 8), and neutropenia (n = 3). Two patients had grade 3 asymptomatic, subsegmental pulmonary embolism. Two subjects did not complete the initial treatment phase: one individual was taken off study after developing grade 2 priapism, and a second individual expired because of pneumonia/neutropenic sepsis. AEs occurring during the BAT

monotherapy phase of the trial were rare and low-grade. Only four subjects experienced an AE during this phase, and all but three AEs were grade 1. Grade 2 events included alopecia and an elevated creatinine in one subject and grade 2 nausea in a separate subject. None of the 14 patients developed new pain, skeletal events, or urinary obstruction due to prostate cancer. Frequently observed (>15%) and severe (grade 3 to 4) AEs are summarized in Table 3. Patients with intact sexual function before ADT had return of sexual function and libido on BAT.

Discussion

This pilot clinical study was performed to test the safety and efficacy of pharmacologic doses of testosterone and oral etoposide in men with asymptomatic CRPC who were progressing on long-term ADT. The study demonstrated that systemic administration of sufficient testosterone to produce supraphysiologic serum testosterone levels was well tolerated in these asymptomatic men with low- to moderate-burden metastatic disease. No patient developed worsening pain due to prostate cancer, nor were there any other skeletal events or evidence of worsening urinary obstruction. Furthermore, with seven patients exhibiting PSA declines, the study met the primary endpoint of at least three subjects with PSA declines. The PSA declines observed in this study are particularly remarkable because PSA is an androgen-stimulated gene product. BAT also produced objective radiographic responses in 50% of patients with RECIST-evaluable disease. Finally, post hoc analysis showed that 10 of 10 (100%) of men treated with BAT responded to second-line therapies after BAT, with 3 subjects responding to an agent on which they had previously progressed. These data suggest that BAT may have the potential to reverse resistance to androgen-ablative therapies, potentially resensitizing men to drugs to which their cancer had become resistant. Although this pilot study enrolled only a small number of patients, it provides compelling preliminary evidence that challenges the current treatment paradigm for CRPC, which is focused primarily on inhibiting ligand binding to AR.

There are three critical aspects of this study design that must be emphasized. First, as an eligibility criterion, men enrolled on this study had to have progressive CRPC treated continuously with ADT for ≥1 year. Preclinical mechanistic studies demonstrated that CRPC cell models, which adaptively overexpress AR, were vulnerable to supraphysiologic testosterone (8, 26, 33–35). Thus, this eligibility requirement was put in place to select patients who were likely to have resistance because of AR overexpression induced by chronic exposure to a low testosterone environment. Indeed, the observation that a high baseline PSA predicted for response to BAT may support the hypothesis that AR-driven prostate cancers are more likely to respond to testosterone-based therapies. On the basis of the proposed mechanisms of action, the use of testosterone in men who have not yet received ADT would be ill advised without definitive clinical evidence of benefit. A number of small case reports have documented the potential for testosterone to induce progression in men who are hormone therapy– naïve, including the first paper by Huggins *et al.*, in which he describes clinical progression in a small number of men treated with an androgen (1, 2, 45, 46).

A second important point that must be stressed is that only asymptomatic men were eligible to enroll in the study. This requirement was put in place based on older literature describing the use of “androgen priming” in conjunction with either ^{32}P or chemotherapy to treat men with CRPC and painful bony metastases. In these studies, testosterone administration produced an acute increase in pain within hours to days, which occasionally required hospitalization. The time course of the described acute pain suggests that the increased pain was due to testosterone stimulation of inflammation and/or cytokine release within sites of bone metastases rather than a direct effect on tumor growth. Over a similar time course, men with pain from bone metastases often have a marked improvement in pain after ADT that can occur within hours of castration, not because of tumor death but more likely caused by a decreased expression of inflammatory cytokines in the microenvironment (2). With this concern in mind, the trial was designed to stop treatment and remove patients from study if pain due to prostate cancer developed. However, in this study, none of the men who were asymptomatic at baseline experienced any bone pain due to prostate cancer after receiving multiple cycles of BAT. These results suggest that treatment with testosterone in this asymptomatic population did not stimulate an inflammatory response sufficient to elicit pain, nor did it stimulate progression of disease sufficient to elicit pain. BAT is, however, in no way ready for widespread adoption and should only be administered in the context of a clinical trial until its safety and efficacy can be confirmed.

Finally, although this is not the first study to test the effect of systemic testosterone in men with prostate cancer, it is designed to evaluate the effects of systemic testosterone administration according to a dose and schedule that produce rapid cycling from supraphysiologic to near-castrate serum testosterone levels over a 28-day cycle. Previous case reports documented responses in men treated with daily injections of various testosterone preparations (45–47). Two phase 1 studies reported the results of the use of transdermal testosterone as therapy for men with CRPC who had minimal to moderate disease burden and no pain due to prostate cancer (48, 49). Between the two studies, 7 of 27 men had at least a 20% decline in PSA, but only 1 achieved a >50% decline. Although the studies were considered “negative” from the standpoint of disease response, in both studies, the administration of parenteral testosterone to men with CRPC was very well tolerated and did not result in notable worsening of disease or symptoms, including pain flares. In each of these phase 1 studies, doses of transdermal testosterone up to 7.5 mg/day only produced eugonadal testosterone levels. These transdermal testosterone preparations are designed to produce sustained eugonadal testosterone levels, and it is difficult to reach supraphysiologic levels with currently approved transdermal testosterone preparations without increasing the percentage of testosterone in the preparation (43, 50). Therefore, we chose to use intramuscular injection of testosterone cypionate at the FDA-approved dose of 400 mg to achieve rapid cycling between supraphysiologic and near-castrate serum testosterone levels. Similarly, in preclinical in vitro and in vivo studies, supraphysiologic levels of androgen are used to achieve an antitumor response (8, 26,33–35). We further demonstrate that exposure to supraphysiologic testosterone can generate DSBs in androgen-starved cells and can prevent degradation of high levels of nuclear AR in mitosis, thereby inhibiting DNA relicensing (26, 37). Thus, CRPC cells that maintain high AR levels will be vulnerable to cell death when suddenly exposed to supraphysiologic testosterone conditions because of an

inability to rapidly down-regulate AR; cells that do manage to survive through basal low-level AR expression or adaptive down-regulation of AR will become vulnerable to cell death when suddenly exposed to the near-castrate testosterone conditions that occur over a cycle of BAT. It is unclear, however, why all cell lines exposed to supraphysiologic androgen concentrations were not growth-inhibited. Future studies are needed to determine why some prostate cancer cell lines are growth-inhibited by androgen and others are not. Specific attention should be paid to evaluating effects of androgen on AR expression, DSB repair, and AR-regulated gene expression changes to better understand this process.

An additional feature of this trial that sets it apart from past studies using systemic testosterone was the incorporation of etoposide during the first three cycles of therapy. Etoposide showed minimal activity against prostate cancer in a previous study and is not used routinely as a treatment for prostate cancer (39). However, as outlined in this report, etoposide may increase and/or stabilize testosterone-induced DSBs, thus providing the motivation to combine BAT and etoposide. Given that four of seven (57%) subjects reached a PSA nadir during the testosterone monotherapy second phase of the trial, as well as the fact that PSA progression typically occurred well after the discontinuation of etoposide, with a median time to PSA progression of 221 days (range, 95 to 454 days), it is unclear if the etoposide contributed to the clinical efficacy observed on this trial. In addition, the substantial toxicity associated with etoposide has tempered our enthusiasm for combining it with BAT. However, based on the potential for mechanistic synergy in inducing transient DSBs, the possibility remains that etoposide may have contributed to the responses observed in this clinical trial. Future studies to separate the contributions of these therapies are warranted.

Although not a prespecified endpoint, the observed high rate of PSA decline with androgen-ablative therapies after BAT raises the possibility that BAT may reverse resistance and resensitize CRPC cells to therapies inhibiting AR signaling pathways. Although these findings need to be further validated in a larger study, this observation could have a major impact on the current treatment paradigm. Recently, data have begun to emerge documenting decreasing efficacy when AR-directed therapies are used sequentially (17–20). This is highlighted by the fact that the reported major PSA response rates (≥50% PSA decline) to enzalutamide after abiraterone or abiraterone after enzalutamide are low, ranging from 3 to 29% (17–20). In this pilot study, 100% of patients demonstrated a PSA decline to androgen-ablative therapies after BAT. This includes three men who received abiraterone before BAT and then had major PSA responses to enzalutamide after BAT, with PSA declines ranging from 53.2 to 99.5%. Similarly, the rate of PSA decline was high upon treatment with abiraterone after BAT, and radiographic responses were robust with the administration of any AR-directed therapy after BAT. The mechanism underlying this sensitization requires further study.

Androgen acutely eliminates AR-V7 expression in the androgen-sensitive VCaP line but has no effect on the AR-V7–expressing CWR22-Rv1 line. In addition, androgen inhibits growth of LNCaP human prostate cancer cells, which completely lack AR-V7 expression. The expression of these AR splice variants has been associated with resistance to ADT, abiraterone, and enzalutamide (27–32). It was recently reported that AR-V7, the best

described AR splice variant, can be found in >35% of men at the time of initiating enzalutamide, with its presence predicting lower PSA response rates (0% versus 52.6%, $P = 0.004$) and shorter time to progression (median progression-free survival: 2.1 months versus 6.1 months, $P < 0.001$) compared to AR-FL (27). It seems plausible that the acute regulation of AR-FL and AR splice variants (such as AR-V7) by androgen may underlie this sensitization effect. Recent work has shown that AR-FL is transcriptionally repressed by liganded AR through the recruitment of LSD1 to an enhancer found in the *AR* second intron and demethylation of H3K4me1,2 (51). Furthermore, AR-V7 expression may be regulated by androgen through an AR-FL feedback mechanism, by which liganded AR decreases the recruitment of splicing factors U2AF65 and ASF/SF2, which have been implicated in AR pre-mRNA splicing to AR-V7 (52, 53). Given the ability of androgen to suppress AR-FL and AR-V7 expression, it is possible that BAT functions to sensitize cells by acutely lowering AR-FL and AR-V7 protein levels. If BAT is proven to suppress full-length and AR variant expression, its incorporation into the prostate cancer treatment paradigm could serve an important role in extending the effectiveness and mitigating resistance to next-generation AR-directed agents like enzalutamide and abiraterone.

In summary, this study provides preliminary evidence that asymptomatic men with advanced CRPC can be safely treated with BAT to achieve rapid cycling between supraphysiologic and near-castrate serum testosterone levels to produce an antitumor effect. Although PSA flares were observed in some men, there were no symptomatic flare reactions with BAT administration, and BAT produced objective radiographic responses in 50% of patients. Although these results are encouraging, it should be emphasized that the results of this pilot study need confirmation before adopting BAT into a clinical setting, and additional clinical trials are essential to determine whether this treatment approach is safe and effective. Because PSA is an androgen-stimulated gene product, its use as a marker of disease response to BAT is inherently problematic. Thus, future studies should use radiographic or survival endpoints. Finally, the results from this pilot study support future work toward establishing BAT as an effective therapy that can improve survival, overcome resistance to androgen-ablative therapies, and meaningfully improve quality of life, functional activity, and sexual function in men with CRPC.

Materials and Methods

Please refer to Supplementary Materials and Methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We wish to thank M. Rosen, S. Dalrymple, and L. Antony for excellent technical assistance and the clinical research nurses and data managers of the Johns Hopkins Prostate Cancer Research Program who supported this study. We also wish to thank the patients who participated in the clinical study. The clinical study is conducted in memory of Bruce Hunsicker, founder of the One-in-Six Foundation.

Funding: Grant funding provided by the One-in-Six Foundation, Akron, OH.

References and Notes

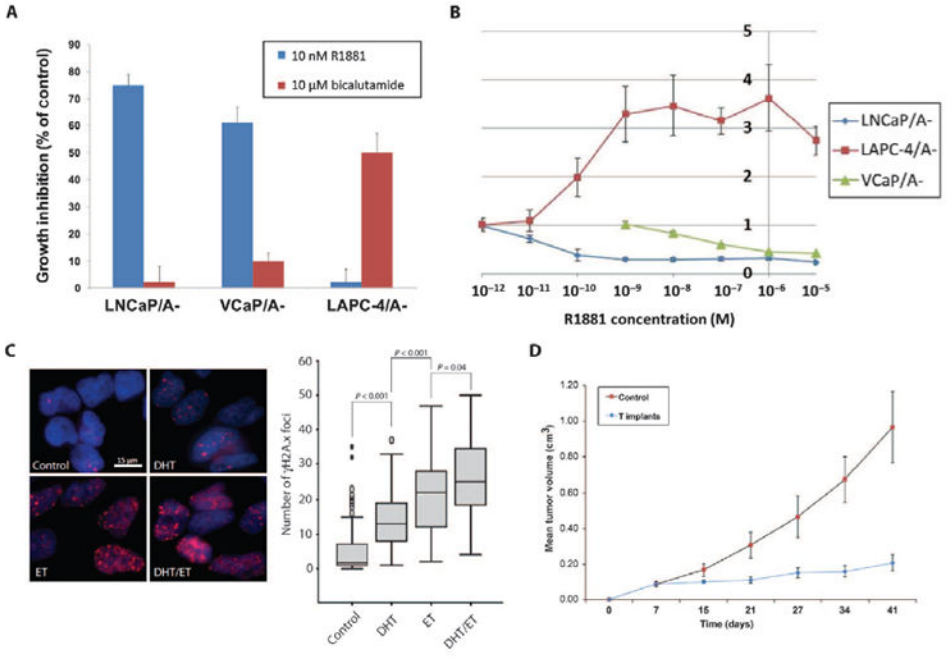
1. Huggins C, Hodges CV. Studies on prostatic cancer. I. The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. 1941. J Urol. 2002; 167:948–951. discussion 952. [PubMed: 11905923]
2. Huggins C, Stevens RE, Hodges CV. Studies on prostatic cancer. II. The effects of castration on advanced carcinoma of the prostate gland. Arch Surg. 1941; 43:209–223.
3. Daniell HW, Dunn SR, Ferguson DW, Lomas G, Niazi Z, Stratte PT. Progressive osteoporosis during androgen deprivation therapy for prostate cancer. J Urol. 2000; 163:181–186. [PubMed: 10604342]
4. Diamond T, Campbell J, Bryant C, Lynch W. The effect of combined androgen blockade on bone turnover and bone mineral densities in men treated for prostate carcinoma: Longitudinal evaluation and response to intermittent cyclic etidronate therapy. Cancer. 1998; 83:1561–1566. [PubMed: 9781950]
5. Smith MR, Lee H, McGovern F, Fallon MA, Goode M, Zietman AL, Finkelstein JS. Metabolic changes during gonadotropin-releasing hormone agonist therapy for prostate cancer: Differences from the classic metabolic syndrome. Cancer. 2008; 112:2188–2194. [PubMed: 18348297]
6. Smith MR, Saad F, Egerdie B, Sieber PR, Tammela TL, Ke C, Leder BZ, Goessl C. Sarcopenia during androgen-deprivation therapy for prostate cancer. J Clin Oncol. 2012; 30:3271–3276. [PubMed: 22649143]
7. Gregory CW, He B, Johnson RT, Ford OH, Mohler JL, French FS, Wilson EM. A mechanism for androgen receptor-mediated prostate cancer recurrence after androgen deprivation therapy. Cancer Res. 2001; 61:4315–4319. [PubMed: 11389051]
8. 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 $p27^{Kip\ 1}$ in androgen-induced cell cycle arrest. Mol Endocrinol. 1998; 12:941–953. [PubMed: 9658399]
9. Mohler JL. Castration-recurrent prostate cancer is not androgen-independent. Adv Exp Med Biol. 2008; 617:223–234. [PubMed: 18497046]
10. Montgomery RB, Mostaghel EA, Vessella R, Hess DL, Kalhorn TF, Higano CS, True LD, Nelson PS. Maintenance of intratumoral androgens in metastatic prostate cancer: A mechanism for castration-resistant tumor growth. Cancer Res. 2008; 68:4447–4454. [PubMed: 18519708]
11. Nacusi LP, Tindall DJ. Androgen receptor abnormalities in castration-recurrent prostate cancer. Expert Rev Endocrin Metab. 2009; 4:417–422.
12. Stanbrough M, Bubley GJ, Ross K, Golub TR, Rubin MA, Penning TM, Febbo PG, Balk SP. Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. Cancer Res. 2006; 66:2815–2825. [PubMed: 16510604]
13. Weber MJ, Gioeli D. Ras signaling in prostate cancer progression. J Cell Biochem. 2004; 91:13–25. [PubMed: 14689577]
14. de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, Chi KN, Jones RJ, Goodman OB Jr, Saad F, Staffurth JN, Mainwaring P, Harland S, Flaig TW, Hutson TE, Cheng T, Patterson H, Hainsworth JD, Ryan CJ, Sternberg CN, Ellard SL, Fléchon 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. [PubMed: 21612468]
15. Ryan CJ, Smith MR, de Bono JS, Molina A, Logothetis CJ, de Souza P, Fizazi K, Mainwaring P, Piulats JM, Ng S, Carles J, Mulders PF, Basch E, Small EJ, Saad F, Schrijvers D, Van Poppel H, Mukherjee SD, Suttmann H, Gerritsen WR, Flaig TW, George DJ, Yu EY, Efstathiou E, Pantuck A, Winquist E, Higano CS, Taplin ME, Park Y, Kheoh T, Griffin T, Scher HI, Rathkopf DE. COU-AA-302 Investigators. Abiraterone in metastatic prostate cancer without previous chemotherapy. N Engl J Med. 2013; 368:138–148. [PubMed: 23228172]
16. 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; 367:1187–1197. [PubMed: 22894553]

17. 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. 2014; 50:78–84. [PubMed: 24074764]
18. Loriot Y, Bianchini D, Ileana E, Sandhu S, Patrikidou A, Pezaro C, Albiges L, Attard G, Fizazi K, De Bono JS, Massard C. Antitumour activity of abiraterone acetate against metastatic castration-resistant prostate cancer progressing after docetaxel and enzalutamide (MDV3100). Ann Oncol. 2013; 24:1807–1812. [PubMed: 23576708]
19. 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; 24:1802–1807. [PubMed: 23585511]
20. 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. 2014; 65:30–36. [PubMed: 23849416]
21. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer. 2009; 45:228–247. [PubMed: 19097774]
22. Attard G, Swennenhuis JF, Olmos D, Reid AH, Vickers E, A'Hern R, Levink R, Coumans F, Moreira J, Riisnaes R, Oommen NB, Hawche G, Jameson C, Thompson E, Sipkema R, Carden CP, Parker C, Dearnaley D, Kaye SB, Cooper CS, Molina A, Cox ME, Terstappen LW, de Bono JS. Characterization of ERG, AR and PTEN gene status in circulating tumor cells from patients with castration-resistant prostate cancer. Cancer Res. 2009; 69:2912–2918. [PubMed: 19339269]
23. Linja MJ, Savinainen KJ, Saramäki OR, Tammela TL, Vessella RL, Visakorpi T. Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. Cancer Res. 2001; 61:3550–3555. [PubMed: 11325816]
24. Shah RB, Mehra R, Chinnaiyan AM, Shen R, Ghosh D, Zhou M, Macvicar GR, Varambally S, Harwood J, Bismar TA, Kim R, Rubin MA, Pienta KJ. Androgen-independent prostate cancer is a heterogeneous group of diseases: Lessons from a rapid autopsy program. Cancer Res. 2004; 64:9209–9216. [PubMed: 15604294]
25. Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, Rosenfeld MG, Sawyers CL. Molecular determinants of resistance to antiandrogen therapy. Nat Med. 2004; 10:33–39. [PubMed: 14702632]
26. 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; 72:1491–1505. [PubMed: 22396319]
27. Antonarakis ES, Lu C, Wang H, Luber B, Nakazawa M, Roeser JC, Chen Y, Mohammad TA, Chen Y, Fedor HL, Lotan TL, Zheng Q, De Marzo AM, Isaacs JT, Isaacs WB, Nadal R, Paller CJ, Denmeade SR, Carducci MA, Eisenberger MA, Luo J. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. N Engl J Med. 2014; 371:1028–1038. [PubMed: 25184630]
28. 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; 68:5469–5477. [PubMed: 18593950]
29. 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. [PubMed: 19117982]
30. 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; 73:483–489. [PubMed: 23117885]

31. Mostaghel EA, Marck BT, Plymate SR, Vessella RL, Balk S, Matsumoto AM, Nelson PS, Montgomery RB. Resistance to CYP17A1 inhibition with abiraterone in castration-resistant prostate cancer: Induction of steroidogenesis and androgen receptor splice variants. Clin Cancer Res. 2011; 17:5913–5925. [PubMed: 21807635]
32. 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; 120:2715–2730. [PubMed: 20644256]
33. 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; 93:11802–11807. [PubMed: 8876218]
34. 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. [PubMed: 17015840]
35. Vander Griend DJ, Litvinov IV, Isaacs JT. Stabilizing androgen receptor in mitosis inhibits prostate cancer proliferation. Cell Cycle. 2007; 6:647–651. [PubMed: 17387277]
36. D'Antonio JM, Vander Griend DJ, Isaacs JT. DNA licensing as a novel androgen receptor mediated therapeutic target for prostate cancer. Endocr Relat Cancer. 2009; 16:325–332. [PubMed: 19240183]
37. Haffner MC, Aryee 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; 42:668–675. [PubMed: 20601956]
38. Haffner MC, De Marzo AM, Meeker AK, Nelson WG, Yegnasubramanian S. Transcription-induced DNA double strand breaks: Both oncogenic force and potential therapeutic target? Clin Cancer Res. 2011; 17:3858–3864. [PubMed: 21385925]
39. Hussain MH, Pienta KJ, Redman BG, Cummings GD, Flaherty LE. Oral etoposide in the treatment of hormone-refractory prostate cancer. Cancer. 1994; 74:100–103. [PubMed: 8004566]
40. 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; 70:1600–1607. [PubMed: 20607766]
41. Sedelaar JP, Isaacs JT. Tissue culture media supplemented with 10% fetal calf serum contains a castrate level of testosterone. Prostate. 2009; 69:1724–1729. [PubMed: 19676093]
42. Chuu CP, Kokontis JM, Hiipakka RA, Fukuchi J, Lin HP, Lin CY, Huo C, Su LC, Liao S. Androgen suppresses proliferation of castration-resistant LNCaP 104-R2 prostate cancer cells through androgen receptor, Skp2, and c-Myc. Cancer Sci. 2011; 102:2022–2028. [PubMed: 21781227]
43. Behre, HM.; Nieschlag, E. Testosterone preparations for clinical use in males. In: Nieschlag, E.; Behre, HM., editors. Testosterone: Action, Deficiency, Substitution. 4. Cambridge Univ. Press; Cambridge, UK: 2012.
44. Swerdloff, RS.; Wang, CCL. Testosterone: Action, Deficiency, Substitution. 4. Cambridge Univ. Press; Cambridge, UK: 2012. Review of guidelines on diagnosis and treatment of testosterone deficiency.
45. Brendler H, Chase WE, Scott WW. Prostatic cancer: Further investigation of hormonal relationships. Arch Surg. 1950; 61:433–440. [PubMed: 15433723]
46. Prout GR Jr, Brewer WR. Response of men with advanced prostatic carcinoma to exogenous administration of testosterone. Cancer. 1967; 20:1871–1878. [PubMed: 4168724]
47. Mathew P. Prolonged control of progressive castration-resistant metastatic prostate cancer with testosterone replacement therapy: The case for a prospective trial. Ann Oncol. 2008; 19:395–396. [PubMed: 18156142]
48. 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; 56:237–244. [PubMed: 19375217]

49. 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; 56:97–103. [PubMed: 19282098]
50. Leibowitz RL, Dorff TB, Tucker S, Symanowski J, Vogelzang NJ. Testosterone replacement in prostate cancer survivors with hypogonadal symptoms. BJU Int. 2010; 105:1397–1401. [PubMed: 19912179]
51. Cai C, He HH, Chen S, Coleman I, Wang H, Fang Z, Chen S, Nelson PS, Liu XS, Brown M, Balk SP. Androgen receptor gene expression in prostate cancer is directly suppressed by the androgen receptor through recruitment of lysine-specific demethylase 1. Cancer Cell. 2011; 20:457–471. [PubMed: 22014572]
52. Liu LL, Xie N, Sun S, Plymate S, Mostaghel E, Dong X. Mechanisms of the androgen receptor splicing in prostate cancer cells. Oncogene. 2014; 33:3140–3150. [PubMed: 23851510]
53. Yu Z, Chen S, Sowalsky AG, Voznesensky OS, Mostaghel EA, Nelson PS, Cai C, Balk SP. Rapid induction of androgen receptor splice variants by androgen deprivation in prostate cancer. Clin Cancer Res. 2014; 20:1590–1600. [PubMed: 24449822]
54. Scher HI, Halabi S, Tannock I, Morris M, Sternberg CN, Carducci MA, Eisenberger MA, Higano C, Bubley GJ, Dreicer R, Petrylak D, Kantoff P, Basch E, Kelly WK, Figg WD, Small EJ, Beer TM, Wilding G, Martin A, Hussain M. Prostate Cancer Clinical Trials Working Group. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: Recommendations of the Prostate Cancer Clinical Trials Working Group. J Clin Oncol. 2008; 26:1148–1159. [PubMed: 18309951]
55. Simon R. Optimal two-stage designs for phase II clinical trials. Control Clin Trials. 1989; 10:1–10. [PubMed: 2702835]
56. Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, Quist MJ, Jing X, Lonigro RJ, Brenner JC, Asangani IA, Ateeq B, Chun SY, Siddiqui J, Sam L, Anstett M, Mehra R, Prensner JR, Palanisamy N, Ryslik GA, Vandin F, Raphael BJ, Kunju LP, Rhodes DR, Pienta KJ, Chinnaiyan AM, Tomlins SA. The mutational landscape of lethal castration-resistant prostate cancer. Nature. 2012; 487:239–243. [PubMed: 22722839]
57. Hu R, Lu C, Mostaghel EA, Yegnasubramanian S, Gurel M, Tannahill C, Edwards J, Isaacs WB, 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; 72:3457–3462. [PubMed: 22710436]
58. D'Antonio JM, Vander Griend DJ, Antony L, Ndikuyeze G, Dalrymple SL, Koochekpour S, Isaacs JT. Loss of androgen receptor-dependent growth suppression by prostate cancer cells can occur independently from acquiring oncogenic addiction to androgen receptor signaling. PLOS One. 2010; 5:e11475. [PubMed: 20628607]
59. Uzgare AR, Isaacs JT. Enhanced redundancy in Akt and mitogen-activated protein kinase-induced survival of malignant versus normal prostate epithelial cells. Cancer Res. 2004; 64:6190–6199. [PubMed: 15342404]
60. Gao J, Arnold JT, Isaacs JT. Conversion from a paracrine to an autocrine mechanism of androgen-stimulated growth during malignant transformation of prostatic epithelial cells. Cancer Res. 2001; 61:5038–5044. [PubMed: 11431338]



E

	Castrate	Castrate + T implants	*P* value
Tumor volume (cm^3)	0.98 ± 0.19	0.32 ± 0.06	0.004
PSA/gram tumor	24.5 ± 8.0	95.5 ± 6.23	2×10^{-5}
Ki-67	83.0 ± 1.8%	81.1 ± 1.5%	0.4
Mitotic index	0.51 ± 0.05	0.32 ± 0.07%	0.04
AR in nucleus	>90%	>90%	**
Cell death index	15 ± 2%	39 ± 3%	1×10^{-5}
AR in mitosis	7 ± 3%	77 ± 3%	1.4×10^{-14}

F

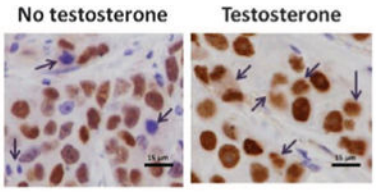
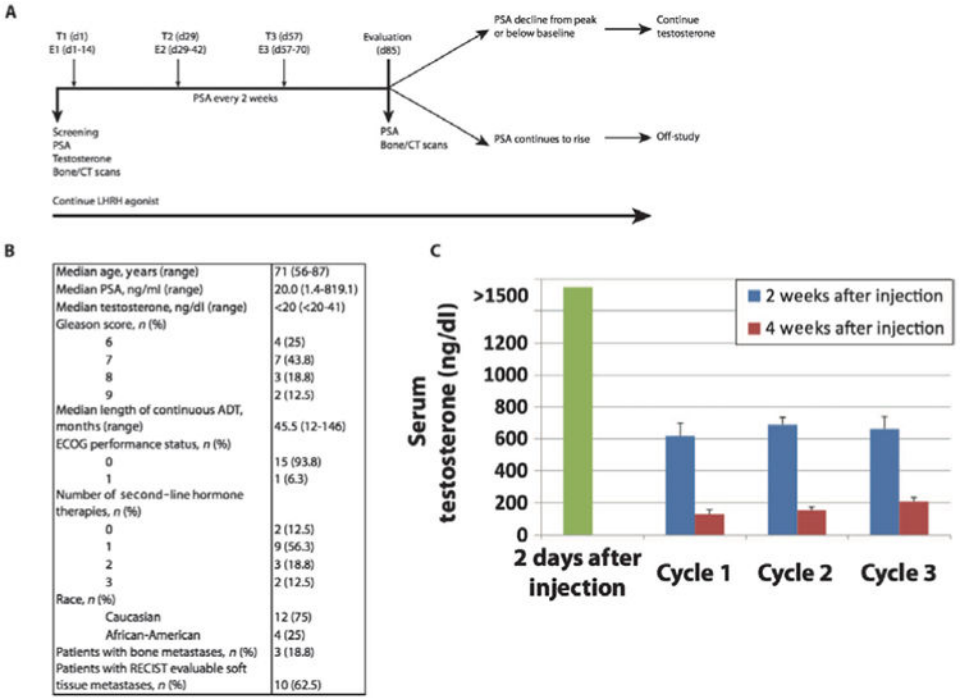


Fig. 1. In vitro and in vivo effect of supraphysiologic androgen

(**A**) Effects of R1881 and bicalutamide on the growth of LNCaP/A–, VCaP/A–, and LAPC-4/A– human prostate cancer cells adapted to grow in media containing charcoal-stripped serum. *Y* axis represents the percentage growth inhibition compared to control (determined by cell count after growth in 0.1% dimethyl sulfoxide–containing medium) after a 5-day exposure to 10 nM R1881 or 10 μM bicalutamide. (**B**) R1881 dose response of LNCaP/A–, LAPC-4/A–, and VCaP/A– human prostate cancer cells adapted to grow in medium containing charcoal-stripped serum. *Y* axis is fold change in cell number compared to day 0 control after a 5-day exposure to the indicated concentration of R1881. Data are means ± SD (*n* = 8 replicates per dose per cell type). (**C**) Sample image (×100 magnification) and quantification of the amount of γH2A.x foci induced in LNCaP cells after exposure to 100 nM DHT or 100 μM etoposide or the combination. *Y* axis is number of foci per cell (*n* = 100 cells counted per treatment condition). Scale bar, 15 μm. (**D**) Growth of LNCaP/A– xenografts in castrated nude mice treated with subcutaneous silastic implants filled with either nothing (Control) or testosterone beginning on day 7 (*n* = 10 animals per group; $P < 0.05$ for all time points beginning at day 21). (**E**) Evaluation of indicated parameters in LNCaP/A– cells growing in castrate mice that were or were not supplemented with subcutaneous testosterone-filled silastic implants (implants placed in animals for 2 weeks, removed for 2 weeks, and then replaced again for 2 weeks before tumor harvest). All

values are presented as means ± SE of representative data generated from one of a minimum of three independent experiments, in which there were a minimum of eight replicates per data point. To determine indices, 200 cells per slide were evaluated for each index to determine percentages. Original data for individual mice can be found in table S1. (**F**) Immunohistochemical staining for AR in harvested LNCaP/A– xenografts growing in castrate mice that were or were not supplemented with subcutaneous testosterone-filled silastic implants. Blue arrows indicate mitotic figures. Scale bars, 15 μm.



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

Fig. 2. Clinical trial of BAT plus etoposide

(**A**) Schematic of study design. (**B**) Baseline characteristics of patients on study. (**C**) Mean serum testosterone levels at indicated time points for patients on study.

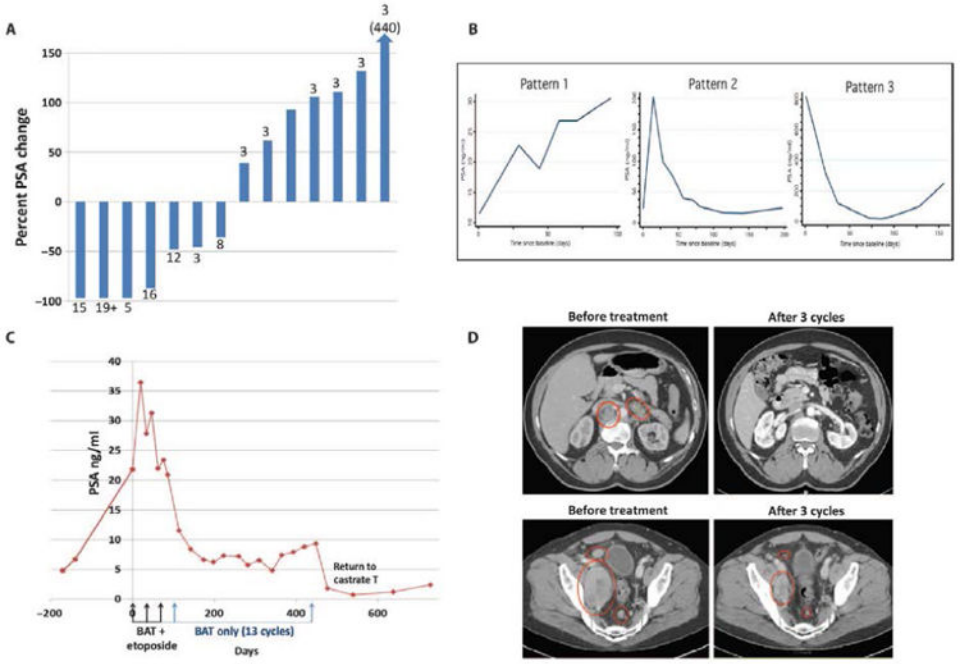


Fig. 3. Evaluation of clinical response

(**A**) Waterfall plot showing maximum PSA change relative to baseline in 14 patients completing at least three cycles of BAT plus etoposide. The number at the end of each bar indicates the number of treatment cycles received. The number in parentheses indicates the percent PSA change in a case where the bar was truncated. (**B**) Patterns of PSA response observed in patients on study. (**C**) PSA response in an individual patient (patient 9) receiving a total of 16 cycles of BAT. (**D**) Computed tomography (CT) scans obtained before treatment and after three cycles of therapy in two patients on study, demonstrating complete response (CR) (patient 16, upper panels) and partial response (PR) (patient 15, lower panels) in abdominal lymph nodes. Red circles outline disease burden.

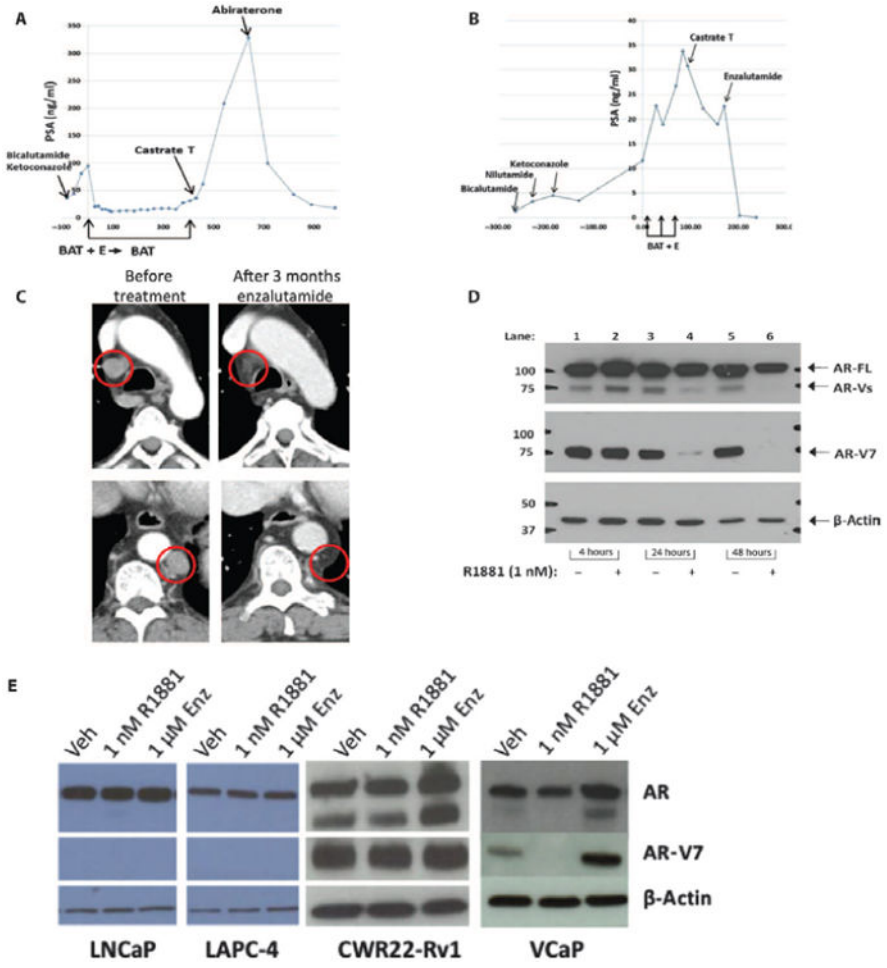


Fig. 4. Response to second-line therapy after BAT

(**A**) PSA response to abiraterone in an individual patient (patient 4) who had received previous therapy with bicalutamide and ketoconazole and had a sustained PSA response over 15 cycles of BAT. (**B**) Major PSA response (>50% PSA decline) to enzalutamide in a patient (patient 14) who received previous therapy with bicalutamide, nilutamide, and ketoconazole and failed to have a PSA response with three cycles of BAT plus etoposide. (**C**) Complete resolution of enlarged lymph nodes (red circles) on CT scan in the same patient 14 after 3 months of therapy with enzalutamide. (**D**) Effect of R1881 (1 nM) on the expression of AR-FL, AR variants, and AR-V7 in VCaP human prostate cancer cells over a 48-hour exposure. (**E**) AR-V7 is not expressed in LNCaP or LAPC-4, two cell lines that are sensitive to growth inhibition by androgens. CWR22-Rv1 cells express high levels of AR-V7, which is not down-regulated by exposure to R1881 or up-regulated upon exposure to enzalutamide. The AR-V7–positive VCaP line demonstrates AR-V7 down-regulation when exposed to R1881 and up-regulation when exposed to enzalutamide.

Sci Transl Med. Author manuscript; available in PMC 2015 July 20.

Table 1

Treatment and response summary for all enrolled patients

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable; ND, not determined; T, testosterone; E, etoposide.

Subject number	Duration of previous androgen deprivation (months)	Total follow-up (days)	Cycles on study	PSA change relative to baseline after initial three cycles	On-study PSA low relative to baseline (%)*	Time from enrollment to PSA progression (days)†	Maximum radiographic response‡ T + E (%)
1	45	91	3	347.6	132.5	—	PD
2	55	203	6	157.1	92.9	—	—
3	66	126	3	254.0	111.1	—	—
4	62	434	15	−86.4	−86.4	262	PR
5	146	127	3	261.2	90.15	—	—
6	22	343	12	−24.6	−47.9	221	PR
7	32	91	3	789.0	439.7	—	PD
8	24	91	3	−46.4	−47.0	113	SD
9	48	455	16	−4.1	−78.0	454	—
10	13	95	3	118.9	12.6	—	SD
11	ND	38	1	NE	NE	—	—
12	ND	35	1	NE	NE	—	—
13	92	609	22§	−96.4	−97.0	289	PR
14	54	95	3	162.9	62.9	—	SD
15	99	192	8	38.7	−36.1	168	PR
16	52	146	5	−96.4	−98.0	95	CR

*In cases where the PSA never fell below baseline, the PSA low was taken to be the lowest PSA value observed after initiating treatment with BAT.

†Time only given for those subjects with a PSA response (decline from baseline).

‡RECIST response only given for subjects with RECIST-evaluable disease.

§Patient remains on study.

Table 2

PSA response to secondary hormonal therapy (HT) upon return to castrate testosterone levels after BAT

NA, not applicable.

Subject number*	Maximum PSA decline upon recastration (%)†	Secondary HT received before study	Secondary HT received after study	Maximum PSA decline upon secondary HT initiation (%)‡
1	NA	Nilutamide, bicalutamide	Nilutamide	−44.3
2	−88.2	Bicalutamide	None	NA
3	−57.4	Bicalutamide	Abiraterone	−94.2
4	No decline	Bicalutamide	Abiraterone	−92.7
5	−52.0	Bicalutamide, ketoconazole	Enzalutamide	−30.4
6	−58.2	Bicalutamide	Bicalutamide	−30.8
7	−73.2	Bicalutamide	Abiraterone	−88.1
8	No decline	Bicalutamide, nilutamide	None	NA
9	−92.5	Bicalutamide	None	NA
10	No decline	Bicalutamide	Abiraterone	−63.8
14	−38.2	Nilutamide, bicalutamide, abiraterone	Enzalutamide	−99.5
15	−35.0	Abiraterone	Enzalutamide	−78.3
16	−68.4	Nilutamide, abiraterone, enzalutamide	Enzalutamide	−53.2

*All of these subjects were treated with a hormonal therapy (consisting of an LHRH analog at a minimum) after BAT and completed at least the initial three cycles of BAT plus etoposide. The two subjects who progressed before cycle 3 and the individual who continues on treatment per protocol are excluded from this table.

†Maximum percentage decline in PSA upon testosterone level falling within the castrate range (testosterone <50 ng/dl) compared to the PSA value at the time of study discontinuation (baseline PSA used for this calculation was the end of BAT PSA value). Value only given if PSA was measured after return to castrate testosterone level and before receiving a secondary HT.

‡Calculation is based on a baseline PSA value at the time of initiating a secondary HT.

Table 3

Adverse events occurring in >15% of subjects (*n* = 16) and severe (grade 3 to 4) events

All of those events listed occurred during the testosterone plus etoposide phase of the trial.

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