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Bipolar AndrogenTherapy: The Rationale for Rapid Cycling of Supraphysiologic Androgen/Ablation in Men With Castration Resistant Prostate Cancer

Samuel R. Denmeade[*] and **John T. Isaacs**
The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland

Abstract

Androgen ablation is highly effective palliative therapy for metastatic prostate cancer but eventually all men relapse. New findings demonstrating that androgen receptor (AR) expression continues in androgen ablated patients has resulted in the classification "Castration Resistant Prostate Cancer" (CRPC) and has led to the development of new second-line "anti-ligand" hormonal agents. In this background is the paradoxical observation that the growth of some AR-expressing "androgen sensitive" human prostate cancer cells can be inhibited by supraphysiologic levels of androgens. This response may be due to effects of high-dose androgen on inhibiting re-licensing of DNA in cells expressing high levels of AR. It may also be due to recently described effects of androgen in inducing double strand DNA breaks. Based on available preclinical data described in this review demonstrating the effects of supraphysiologic levels of testosterone on inhibition of growth of CRPC xenografts, we initiated a clinical trial in men with CRPC testing the effect of monthly treatments with an intramuscular (IM) depot injection of testosterone. This IM formulation achieves supraphysiologic levels of testosterone that cannot be achieved with standard testosterone gel-based applications. The supraphysiologic testosterone level is followed by a rapid drop to castrate levels of testosterone with each cycle of therapy. This "bipolar androgen therapy" will not allow time for prostate cancer cells to adapt their AR expression in response to environmental conditions. The goal is to determine if a clinical response can be achieved through this non-adaptive rapid cycling approach in men with CRPC.

Keywords

castration-resistance; androgen receptor; clinical trial; etoposide

INTRODUCTION

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

[*]Correspondence to: Samuel R. Denmeade, MD, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, The Bunting Blaustein Cancer Research Building, 1650 Orleans Street, Baltimore, MD 21231. denmesa@jhmi.edu.

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, in the majority of prostate cancer specimens from androgen ablated patients, a subset of the prostate cancer cells continue to express the androgen receptor (AR) [5,6]. The prostate cancer cells from these refractory patients also 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 some “castration-resistant” prostate cancer cells may continue to survive through aberrant AR signaling. This observation has led to a renewed interest in the AR axis as a therapeutic target. On this basis a number of new more potent antiandrogens and androgen synthesis inhibitors are currently undergoing testing in large phase III trials. Alternative strategies that can directly lower the AR protein level (e.g., siRNA, HSP90 inhibitors) have been demonstrated to consistently inhibit growth of cells expressing either wild-type or ligand-independent AR variants and theses approaches are also being explored clinically [7,8].

In this background of renewed interest in blocking AR, there has been the paradoxical observation that AR expressing “androgen sensitive” human prostate cancer cell lines grow optimally in serum containing growth media that contains castrate levels of androgen [9]. The growth of these AR expressing prostate cancer cells can be inhibited by the addition of exogenous androgens into the growth media [10–15]. Growth inhibition by exogenous androgens is also observed when AR negative prostate cancer cells are transfected with the AR gene [16–18]. Androgen levels in this case can be as low as picomolar concentrations of synthetic androgens suggesting that these cells can be exquisitely sensitive to androgens. Remarkably, in these studies antiandrogens such as bicalutamide are able to “rescue” these AR expressing cells from androgen growth suppression. These in vitro studies are supported by animal studies demonstrating that AR positive human prostate cancer cells selected to grow in a castrate host can upregulate AR levels [19–21]. In these in vivo studies, administration of supraphysiologic systemic testosterone (T) produces significant growth inhibition, whereas antiandrogens promote prostate cancer growth [19,20]. Anecdotal evidence in humans treated with T supports these animal model observations [22–25].

The goal of this review is to summarize the preclinical data and clinical experience on the effects of androgens in castrate-resistant prostate cancer cells in an effort to support further clinical testing of this approach in men with CRPC.

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). In the United States, these therapies include either surgical castration or medical castration with LHRH agonists. All men treated with standard castrating therapy

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. Over time 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 glands, and perhaps the prostate cancer cells themselves, have the biochemical machinery necessary to synthesize testosterone from steroidal precursors [26–28]. In the initial studies by Huggins, adrenalectomy led to significant improvement in clinical symptoms and declines in acid phosphatase in a subset of men with prostate cancer previously treated with castrating therapies [26]. Currently, the initial standard treatment for men classified as CRPC on the basis of rising PSA is usually the antiandrogen bicalutamide (i.e., Casodex®), followed by other antiandrogens such as nilutamide (i.e., Nilandron®) and, eventually, treatment with the adrenal androgen synthesis inhibitor ketoconazole [29]. These agents are associated with a PSA response rate (i.e., PSA decreases by 50% from baseline level) of 10–40% with higher response rates observed overall for ketoconazole across studies versus the antiandrogens [29]. The impact of these second-line hormonal therapies on overall survival is unknown and untested. Currently, an ongoing randomized phase III study of a new hormonal agent, abiraterone acetate, will be the first trial to assess whether a secondary hormonal manipulation can impact patient survival [30]. A second agent, MDV3100, is also undergoing testing in Phase III studies with similar survival endpoints [31].

The accepted rationale for the use of second-line hormonal therapies is that the castrate-resistant prostate cancer cells become supersensitive to the low level of circulating androgens. This rationale is supported by observations from an autopsy study that evaluated AR expression in a large number of prostate cancer specimens taken from men with CRPC [6]. In this study AR expression varied across tumor samples with 31% (83 of 265) of the cells within individual tumor samples expressing >50% AR and 41.5% (100 of 265) expressing <10% AR. Overall the median percentage of cells expressing AR within a metastatic site was ~20% (range, 0–100%, SEM, 34.28) in this study. In addition to these autopsy findings, several clinical studies have used FISH analysis to evaluate AR gene copy number in circulating tumor cells (CTCs) and bone marrow metastases from men with CRPC and have documented AR amplification in as many as 50% of patients [32–35].

Further support for the adaptation hypothesis comes from studies by Chen et al. [21] who demonstrated that AR expressing human prostate cancer cell lines readily adapt both in vitro and in vivo to low androgen conditions through increased expression of AR. Prostate cancer cells adapted to low androgen levels in this study became resistant to growth inhibition by standard concentrations of bicalutamide and were growth stimulated by high concentrations of bicalutamide, flutamide, or cyproterone acetate. The data from this study further support the hypothesis that increased AR expression under low androgen conditions may be a cause of the hormone-sensitive to hormone refractory transition. This study did document that low androgen adapted cells still required androgen, but could grow at lower androgen

concentrations then unadapted cells [21]. However, the growth response of these adapted cells to higher dose androgen was not assessed in this study.

The data from Chen et al. and earlier data using AR-positive cell lines in vitro and in vivo supports the hypothesis that AR-expressing prostate cancer cells can adapt to the low androgen environment through upregulation of AR expression. These earlier observations formed the basis for clinical testing of intermittent androgen withdrawal (IAW) in men with prostate cancer [36]. With IAW therapy, men with prostate cancer receive medical castration to lower serum testosterone levels. Once a maximum PSA response is observed, the medical castration therapy is discontinued and the patient followed until PSA begins to rise again at which point patients are treated again with castrating therapy. The major problem with this approach is that most men who receive castration therapy do not rapidly return to eugonadal levels of serum testosterone upon cessation of castrating therapy. Typically it can take months or longer, particular in elderly men. Therefore, just as the prostate cancer cells have time to adapt intracellular AR levels to the castrate levels of serum testosterone, these cells have adequate time to readapt AR levels in response to a slow increase in serum testosterone that occurs over months. Therefore, while IAW may be a preferred method for androgen ablation due to decreased side effects of prolonged castration-induced metabolic syndrome, IAW is unlikely to produce enhanced efficacy compared to chronic castration as both therapies allow for cell adaptation to environmental conditions.

This conclusion is supported by the recent publication of the results of a randomized study conducted by the South European Uroncological Group [37]. In this phase III randomized study, 626 patients who initially responded to androgen ablation with a drop in PSA received castrating therapy with an LHRH analog plus the antiandrogen cyproterone acetate either continuously or intermittently. Intermittent patients received therapy to drive PSA down to <4 ng/ml at which point therapy was stopped until PSA climbed to >10 ng/ml for symptomatic or >20 ng/ml for asymptomatic patients. Although patients in the IAW arm experienced fewer side effects, after a median follow-up time of 51 months there was no survival benefit in favor of either arm. Of note, for patients in the IAW arm, 50% were off therapy for at least 52 weeks following the initial LHRH therapy and 29% were off therapy for >36 months. For the 197 patients on the IAW arm whose PSA level went down to <2 ng/ml, the median time off therapy was 74 weeks. When these patients returned to therapy, they had a median of 14 weeks of treatment, followed by a second period off therapy (median: 70 weeks).

PRELIMINARY DATA IN SUPPORT OF BIPOLAR ANDROGEN THERAPY IN CRPC: IN VITRO STUDIES

All of the available “androgen sensitive” human prostate cancer cell lines were initially derived from samples from men with CRPC. Most of the in vitro data on the effect of androgen on the growth of “hormone-refractory” prostate cancer cells comes from studies using the “androgen sensitive” human prostate cancer cell line LNCaP or its various subclones [10–15]. This line has a mutated AR and produces relatively high levels of PSA at baseline with increased production in response to androgen stimulation. While it grows in vivo in an intact host, it can be easily selected to grow in a castrated host. It has been known

for some time that LNCaP cells can exhibit a biphasic response to androgen supplementation when grown in standard tissue culture media containing 10% fetal bovine serum and castrate baseline levels of androgen [9]. The addition of low levels of supplemental androgen to the culture media of these cells causes a slight increase in proliferation and PSA production. Paradoxically, higher levels of androgen, suppresses the growth of these so-called "androgen-sensitive" cells lines. With serial passage in androgen deficient media, these LNCaP 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. This in vitro adaptation to low androgen conditions mimics the disease course in men with CRPC.

While LNCaP cells exhibit the most dramatic growth inhibition in response to androgens, such growth inhibition is also observed with other human prostate cancer cell lines in vitro [38]. We recently compared the effects of androgen on the growth of an additional panel of AR-positive, prostate-cancer cells (Fig. 1). In this assay, androgen profoundly inhibited the growth of LNCaP cells, but also had an effect on VCap and a slight effect on the mouse derived Myc-Cap line. However, this growth inhibition is not universal and some lines, such as LAPC-4, which contains wild-type AR, exhibit the expected growth stimulation in the presence of androgens. However, as documented by Chen et al. [21] this cell line, when serially adapted, also grows well at much lower androgen conditions compared to unadapted cells.

The final set of data demonstrating androgen growth suppression comes from studies in which non-AR expressing prostate cancer cells are transfected with AR to induce AR expression [16–18]. Like the LNCaP cell line, these AR-transfected cells demonstrate dose-responsive growth inhibition when exposed to increasing concentrations of androgen in the culture media (Fig. 2).

IN VIVO STUDIES

In vivo studies using low androgen adapted LNCaP human prostate cancer xenografts have also documented that growth inhibition can be achieved through treatment with exogenous androgens. For example, Chuu et al. [20] documented that xenografts derived from an androgen adapted LNCaP cell line grew well in intact (i.e., non-castrated) nude mice in the absence of exogenous testosterone. However, treatment with testosterone via an implanted pellet increased blood testosterone to supraphysiologic levels (i.e., fourfold higher than testosterone level in intact mice) and resulted in rapid and sustained regression of tumors (Fig. 3). In this study, continued treatment with testosterone resulted in eventual tumor re-growth after ~100-day exposure. Interestingly, in the tumors that had started to re-grow under the influence of testosterone, the return to the castrate state via removal of the testosterone implant resulted in complete cessation of tumor growth [20]. This result suggests that testosterone cycling could result in sustained response in this model.

Analysis of tumor tissue from these various groups demonstrated that tumors formed from unadapted cells produced low level of AR growing under normal conditions in intact mice.

Adapted tumors that had been growing in vitro under conditions of decreased testosterone exhibit significant increase in the level of AR message and protein (Fig. 3) [20]. 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 (i.e., in androgen sensitive cells) (Fig. 3) [20].

ANDROGEN RECEPTOR AS A LICENSING FACTOR

Androgen binding to the AR stabilizes the receptor and results in translocation to the nucleus. In a recent review [42], a collection of studies show how AR may function as a "master regulator of G1-S phase progression," capable of inhibiting p27, activating key cyclin/CDK complexes, while promoting Rb phosphorylation/inactivation to drive prostate cancer cells into S-phase [7,43,44]. In addition to driving cellular proliferation as a transcription factor, studies suggest a role for AR as a licensing factor for DNA replication in cancer cells [45]. Both in vitro and in vivo analyses demonstrated that AR appears to be proteolytically degraded during mitosis in actively dividing prostate cancer cells [20]. Following up on this observation, forced AR over-expression in AR negative PC3 and AR mutant CWR22Rv1 human prostate cancer cells leads to AR stabilization in mitotic cells, resulting in cell cycle arrest and reduced rates of cellular proliferation [18].

Thus, the logic is such that without the timely and complete degradation of AR during mitosis, origins of DNA replication remain AR-bound therefore stalling DNA re-licensing. At normal AR levels, cancer cells are able to fully degrade nuclear/DNA-bound AR allowing for successful progression through mitosis either into G1 to enter another round of cell division or to exit the cell cycle. However, at the higher cellular levels of AR that are seen in prostate cancer cells from men with CRPC, acute elevation in androgen levels produced by testosterone therapy causes sufficient stabilization of DNA bound AR protein to a point where it is not degraded sufficiently during mitosis. Lowering AR protein levels by experimentally techniques such as siRNA under these conditions can result in tumor response [7]. Excessive ligand-dependent stabilization thus results in a fraction of AR protein remaining associated with origins of replication sites, which were licensed and used during the previous cell cycle, such that these origins of replication cannot re-license in G1 of the subsequent cell cycle in the daughter cells. Although this situation allows the daughter cells to progress into S-phase, it prevents them from completely replicating the full content of their genomic DNA, which induces early S-phase growth arrest [18].

These results have led us to hypothesize that during chronic androgen deprivation therapy (ADT), the cellular AR protein levels are slowly upregulated to compensate for the diminished ligand. When ADT is stopped, the rate at which tissue androgen levels return determines whether adaptive changes have sufficient time to down-regulate the elevated level of AR to prevent re-licensing problems. Increasingly in the clinic, men are treated with an intermittent ADT approach in which the ADT is held following a PSA response and testosterone levels are allowed to rise slowly with the eventual recovery of testicular function. However, under these conditions, prostate cancer cells have time to adapt to the slow recovery of serum testosterone.

These data suggest that the efficacy of ADT might be enhanced by a “bipolar androgen therapy” in which chronic ADT is interrupted by cyclic administration of pharmacologically high doses of testosterone given acutely for only a limited period to achieve supraphysiologic serum testosterone levels followed by an abrupt return to castrate levels. In this way, there is insufficient time to completely down-regulate AR during each androgen restoration cycle resulting in DNA replication re-licensing problems that should inhibit the growth of the prostate cancer cells.

ANDROGEN PRODUCES DOUBLE STRAND BREAKS IN HUMAN PROSTATE CANCER CELL LINES

Recent data demonstrate that replenishment of androgen to androgen starved prostate cancer cells can also produces significant double strand DNA breakage that can result in chromosomal and gene rearrangements that include generation of the TMPRSS2-ERG fusion [46]. In a related study, Ju et al. have shown that estrogen signaling in breast cancer cells involves the co-recruitment of estrogen receptor and topoisomerase II beta (TOP2B) to estrogen receptor target sites, where TOP2B introduces transient double strand breaks [47]. Recent evidence suggests that androgen similarly induces TOP2B-mediated double strand breaks at AR target genes [48]. Thus, we hypothesize, based on this observation, that at high doses of androgens, such breaks may persist and ultimately lead to growth suppression. Treatment of androgen-repleted 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 in treated cells [48]. 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. These observations provide the rationale for an ongoing clinical trial testing the efficacy of concurrently administered intramuscular (IM) testosterone and oral etoposide in men with CRPC.

CLINICAL EXPERIENCE WITH TESTOSTERONE IN PROSTATE CANCER

Up until recently, there had been very limited clinical experience in the PSA-era treating CRPC patients with testosterone. Brendler et al. [22] 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. 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 [23] 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. In the long-term castrate in relapse group, five patients received testosterone for at least 1 month and four of five 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 5U. Two case reports exist in the literature. One by Pearson describes a man with advanced prostate cancer who had

responded to orchiectomy for several years and hypophysectomy for 5 months who was then given testosterone and had a "striking fall in serum acid phosphatase, a rise in hemoglobin, and symptomatic improvement," (Fig. 4) [24]. More recently, Mathew [25] reported on the use of testosterone gel replacement therapy in a man with CRPC and observed a sustained decrease in PSA that lasted for approximately 1 year.

Recently, 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) [49]. 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 [50]. They observed no grade 3 or 4 toxicities and no pain flares. Average serum testosterone levels were within normal limits on this study even though the goal was to try to achieve supraphysiologic levels. No objective responses were observed. Four patients had declines of PSA of at least 20% and 1 patient out of 12 achieved a >50% decline in PSA.

In summary, these combined results suggest that systemic testosterone can be administered safely to men with CRPC and minimal disease burden. PSA declines were observed in some of the patients on these two studies, but only 1 patient out of 27 had a reported >50% decline in PSA. Neither of these studies achieved the supraphysiologic levels of testosterone that were used in the in vivo mouse studies. These levels can be achieved in humans with IM testosterone depot-based therapy.

CONCLUSIONS

The available preclinical data suggest that a subset of CRPC may respond to treatment with androgen. This response may be due to effects of high-dose androgen on inhibiting re-licensing of DNA in cells expressing high levels of AR. It may also be due to recently described effects of androgen in inducing double strand DNA breaks which may result in either growth inhibition or activation of cell death. The published clinical data demonstrate that testosterone can be administered to men with CRPC and low disease burden. These studies did not demonstrate significant clinical responses, suggesting that administration of testosterone to achieve physiologic testosterone levels is, by itself, not sufficient to achieve a clinical effect in men with CRPC. Based on animal data showing effects of supraphysiologic levels of testosterone on inhibiting growth of castrate-resistant prostate cancer xenografts, we have initiated a clinical trial in men with CRPC with rising PSA and minimal metastatic

disease (≤5 sites of disease) testing the effect of "bipolar androgen therapy." In this approach, men with CRPC remain on castrating therapy but also receive monthly treatments with an IM depot injection of testosterone. This IM formulation achieves supraphysiologic levels of testosterone that cannot be achieved with standard testosterone gel-based applications. This high-dose serum testosterone level is followed by a rapid drop to castrate levels of testosterone with each cycle of therapy. Concurrent with the testosterone injection, men will receive oral etoposide based on laboratory findings showing the ability of etoposide to augment and stabilize androgen-induced DNA double strand breaks. Additional laboratory studies are underway to assess the effect of combinations of other inhibitors of DNA repair with androgen in an attempt to identify promising regimens that can be tested in clinical trials.

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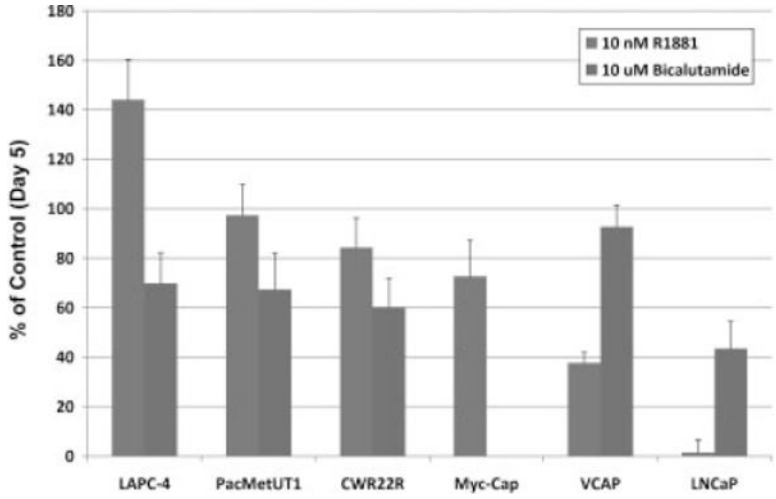

Fig. 1.

Effect of R1881 and bicalutamide on the growth of indicated androgen receptor positive cell lines after 5 days exposure. PacMetUT1 was provided by Dr. De Graffenried (U. Texas Health Science Ctr.), Myc-Cap by Dr. Charles Sawyers (Memorial Sloan Kettering) and VCap by Dr. Ken Pienta (U. Michigan). These lines have been previously described [39–41]. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

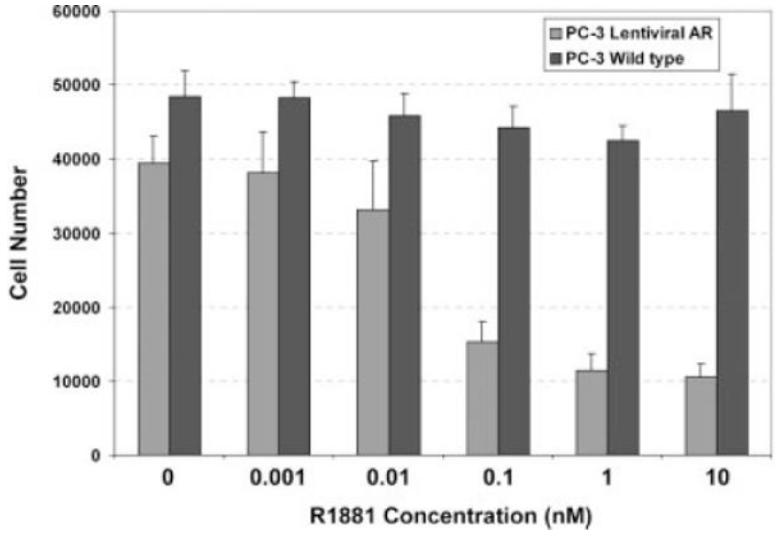

Fig. 2.

Effect of increasing concentrations of R1881 on the growth of wild type PC-3 or PC-3 cells infected lentivirus containing AR gene. Details of this lentiviral construct can be found in Ref. [17]. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

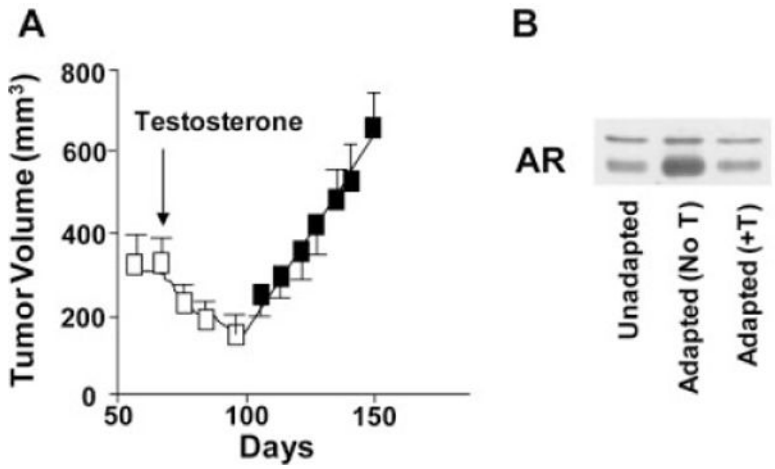

Fig. 3.

Invivostudies with testosterone therapy in LNCaP human prostate cancer xenografts. **A**: Adapted cells (open squares) grow well in castrate animals but rapidly regress upon exposure to supraphysiologic testosterone (T, closed circles), even though tumors are large (~500 – 600 mm^3). Adapted cells growing in castrate animals regress when treated with testosterone (closed squares) but begin to grow with sustained testosterone therapy after ~100 days. **B**: Protein levels of AR show baseline AR levels in non-adapted intact animals. Cells adapted to grow in low testosterone and castrate animals have increased levels of AR. Cells that have begun to re-grow in presence of T show AR levels that have returned to baseline (adapted from Chuu et al. [20]).

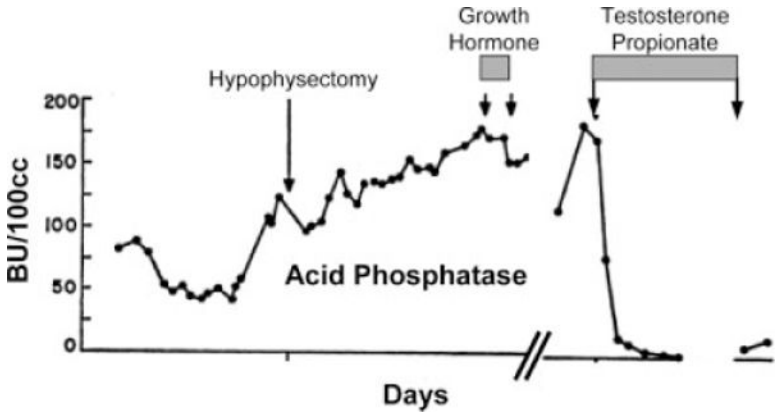

Fig. 4.

Testosterone treatment and acid phosphatase response adapted from Pearson case report [24]. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]