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RAPID COMMUNICATION

Directed Evolution Restored Castrate Sensitivity in a Patient With Castrate Resistant Metastatic Prostate Cancer

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Received: 2 May 2025 | **Revised:** 16 July 2025 | **Accepted:** 20 August 2025

Funding: This study was supported by NIH grant U54-CA274507.

Keywords: cancer evolution | cancer mathematical models | castrate resistant prostate cancer | castrate sensitive prostate cancer | directed evolution

ABSTRACT

Objective: For centuries, humans have used directed evolution to promote desired traits in domesticated animals. We hypothesized similar strategies may be employed to steer castrate resistant prostate cancer cells to a castrate sensitive phenotype allowing resumption of Androgen Deprivation therapy (ADT) and prolonging survival.

Methods: Our interdisciplinary team investigated directed evolution to restore castrate sensitivity in a patient with metastatic castrate resistant prostate cancer who could not tolerate available therapeutic agents for castrate resistant disease. Guided by an evolutionary mathematical model and using the PSA/testosterone ratio as a biomarker for intra-tumoral population dynamics, we applied a sequence of testosterone injections as evolutionary selection forces to suppress resistant androgen receptor–upregulated clones and promote proliferation of ADT-responsive clones.

Results: When the PSA/testosterone ratio indicated successful transition to dominant castrate sensitive population, reinstitution of adaptive dosing of ADT has resulted in three stable cycles.

Conclusion: This case suggests that evolution-informed strategies using population-based biomarkers to manipulate intra-tumoral evolution can restore castrate sensitivity in select patients.

1 | Introduction

Despite increasing numbers of effective treatment agents, metastatic prostate cancer (mPC) remains almost always fatal [1]. First-line therapy includes continuous androgen deprivation therapy (ADT) often with the addition of abiraterone or second-generation androgen receptor signaling inhibitors (ARSIs) [2]. Hormonal treatment is initially highly effective, with serum prostate-specific antigen (PSA) returning to normal or undetectable in over 90% of patients. However, ADT (with or without abiraterone) is virtually never curative. Evolution of resistance typically manifests after 2 to 4 years of treatment. These Darwinian dynamics result in a transition from metastatic castrate-sensitive prostate cancer (mCSPC) to a castrate-resistant state (mCRPC), which confers a poor prognosis (median survival < 2 years) [3].

The critical role of evolution in tumor progression and patient death has motivated investigations into the Darwinian dynamics of tumor progression. As understanding of these dynamics increases, several strategies to delay or prevent proliferation of resistant populations have emerged [4]. One such

strategy, termed "adaptive therapy" [5], has been shown to delay emergence of resistance and prolong tumor control and overall survival [6, 7].

An ongoing adaptive therapy study for metastatic castration sensitive prostate cancer (mCSPC) [8] [NCT03511196] used cycling ADT similar to a prior successful trial using abiraterone in mCRPC [6] The trial has met enrollment goals but, with a median follow-up of 62 months, has not reached the median time to progression. An analysis of patients who have progressed revealed that an increasing ratio of PSA/testosterone corresponds was observed during measurements preceding PSA progression [9]. Prior investigations have demonstrated mPC cells commonly adapt to ADT by increasing the expression of androgen receptors (AR). This hyper-AR expressing phenotype can proliferate at castrate levels of androgen. However, physiological and supraphysiological concentrations of androgens, can elicit a hyperstimulatory state which induces apoptosis [10, 11].

This suggested a strategy that could be implemented during the transition from mCSPC to mCRPC. That is, when PSA progression is observed during ADT, administration of supraphysiological doses of testosterone could promote a population phase change by increasing proliferation of the mPC cells responsive to ADT (termed T +) and inducing cell death in the ADT-resistant cells that upregulate androgen receptors (termed AR + +). We hypothesize that ADT evolves the population from ADT-sensitive to hyper-AR cancer cells, and supraphysiological testosterone provides a mechanism for directed evolution with return to a castrate sensitive state.

The feasibility and safety of testosterone in patients with mPC has been demonstrated in clinical trials using Bipolar Androgen Therapy (BAT) for treatment of mCRPC [12]. These studies demonstrated supraphysiological levels of testosterone could achieve cell death in the castrate resistant phenotype. BAT treatment with regular on/off cycles of testosterone is an effective treatment in mCRPC [13]. Furthermore, after progression on BAT, some patients can again be treated with androgen deprivation therapy [14]. This renewed sensitivity to ADT suggests that, under some circumstances, BAT therapy caused a population transition in which the AR + + cell population has declined, and the T+ phenotype has regained dominance. have regained dominance. Here, we use testosterone injections to direct evolution, guided by the PSA/testosterone ratio, to enable transition from mCRPC to mCSPC, allowing durable tumor control with hormone therapy.

2 | Case Report

The patient is a 73-year-old male with a prior medical history of a BRCA2 germline mutation and familial neuropathy with tremor, gait difficulties, peripheral neuropathy, and hearing loss. In September 2020, the elevation of serum PSA led to a prostate biopsy demonstrating adenocarcinoma, Gleason score 4 + 4, in 7 of 12 bilateral biopsy sites with perineural invasion. Scans revealed metastasis in the left inferior pubic ramus.

Initial therapy was radiation treatment of the prostate plus IMRT of the bony metastases. This was immediately followed by continuous androgen deprivation therapy for 18 months starting in July 2022. Upon completion of adjuvant ADT, serum PSA began to increase. A PSMA-PET scan 6 months after adjuvant-therapy completion demonstrated metastatic disease in the para-aortic lymph nodes, left acetabulum, and right ischium. He began an adaptive therapy regimen using evolution-informed cycling ADT (Orgovyx) like the protocol used in [NCT03511196]. This strategy, termed "adaptive therapy [5], " uses short applications of ADT with the goal of maintaining a significant population of treatment-sensitive cells [7]. After a PSA decline to about ½ of the pretreatment value, ADT is stopped. This allows the tumor population to increase, but, because there is no selection for the resistant phenotype, the sensitive cells will proliferate at the expense of the resistant cells, which retain the "cost" of the resistance molecular machinery while receiving no benefit. Thus, during the off cycle, treatment sensitive cells proliferate while suppressing the resistant population so that, when ADT is resumed after the PSA returns to its pretreatment, the initial favorable response is replicated [6]. The cycles then continue over time producing a stable oscillating population. While this strategy resembles "intermittent therapy" critical differences include absence of a 9 month induction period in which ADT applied continuously at MTD (thus eliminating most of the treatment sensitive cells which are needed to control the resistant cells) and fixed on/off treatment times (e.g., 3 months each) rather than flexible schedules based on the intrinsic rates of population decline during treatment and increase when therapy is withdrawn [15].

Unfortunately, our patient had received 16 months of ADT at MDT and, as seen in Figure 1, during three cycles of adaptive dosing, the PSA troughs increased and the treatment intervals shortened as the PSA/testosterone ratio at the trough of each cycle increased. Addition of abiraterone was attempted but discontinued after 1 week due to intolerable side effects.

In March 2024, PSA increased while on ADT (Figure 2) indicated progression to castrate-resistant disease (mCRPC). The patient declined multiple potential treatments for mCRPC

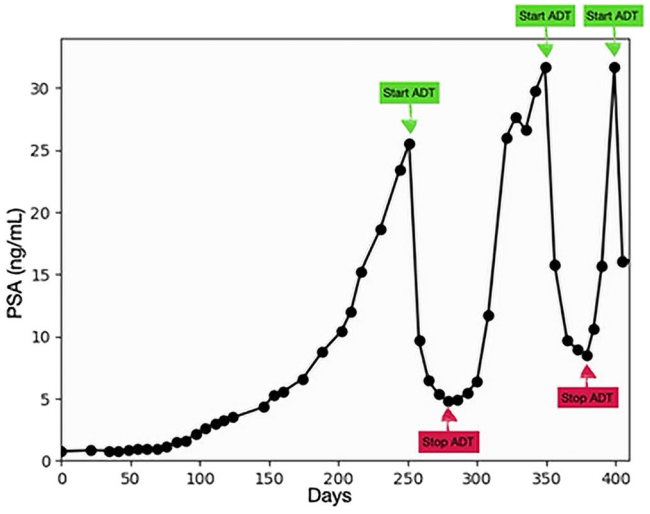

FIGURE 1 | Initial adaptive cycles of ADT following 18 months of adjuvant therapy with continuous ADT at maximum tolerated dose. [Color figure can be viewed at wileyonlinelibrary.com]

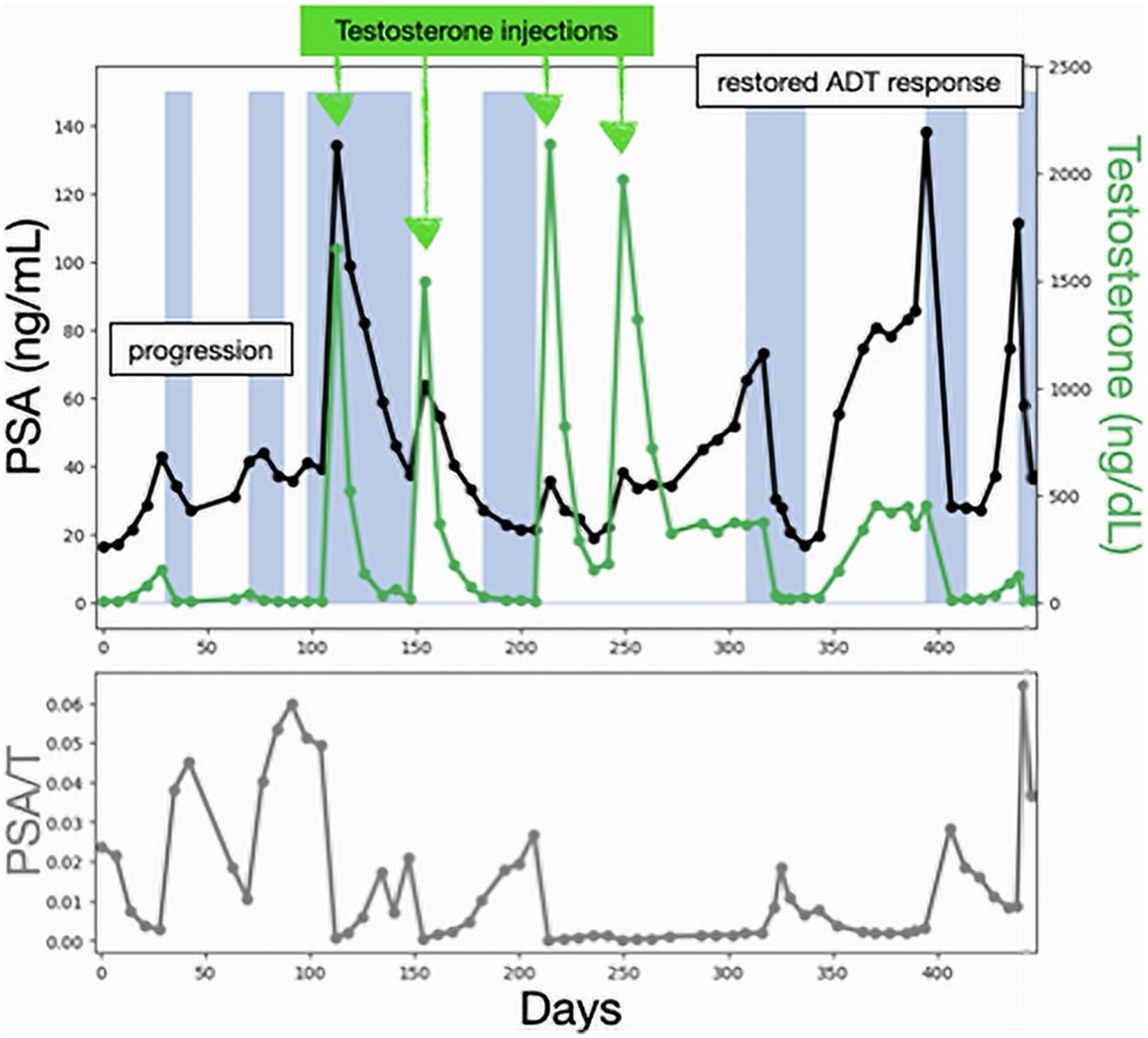

FIGURE 2 | Four cycles of testosterone injection were administered post progression with the goal of increasing fitness of mPC cells that require exogenous testosterone (and sensitive to ADT) while decreasing the fitness of resistant cells that upregulate expression of androgen receptors. Subsequently, rapid responses to ADT in three cycles indicate return to a mCSCPC state. Tumor progression while on ADT coincides with a fluctuating but general increase in the average PSA/testosterone ratio suggesting an increase in size of the resistant population. The testosterone injections were followed by a reduction in the PSA/testosterone ratio fluctuations. [Color figure can be viewed at wileyonlinelibrary.com]

including abiraterone, docetaxel, taxanes, and carboplatin because of concerns for increasing his underlying neuropathy. This led to the decision to engage in directed evolution to restore a castrate-sensitive state using a modified version of BAT.

The patient subsequently underwent four cycles initiated by injection of testosterone cypionate 400 mg IM (Figure 2). Injections were used to generate rapid increases thus minimizing the time available for adaptation by the AR++ cells. In addition, since low levels of testosterone levels applies positive selection for the AR++ population, each dose of testosterone was administered immediately after the serum concentration reached baseline levels following the prior injection. Each injection resulted in a corresponding increase in PSA. The peak PSA value decreased with the initial three cycles but increased slightly in the 4th cycle. The PSA/testosterone ratio decreased in the troughs of the 3rd and 4th cycles compared to the final cycle of ADT and the first two cycles of testosterone. This was interpreted as a population shift to a dominant castrate-sensitive population. Adaptive ADT was reinstituted with three subsequent cycles showing rapid PSA response following administration of Orgovyx (Figure 2). Castrate-sensitivity was restored.

2.1 | Evolutionary Analysis

We hypothesized that, during the transition from mCSPC to mCRPC there are two competing intra-tumoral subpopulations: (1) T+ cells that require physiological levels of testosterone and respond to ADT and (2) AR++ cells that upregulate expression of androgen receptors and, therefore, remain proliferative at castrate testosterone levels. Since the AR++ cells can remain proliferative at low testosterone concentrations, they replace the T+ cells during the ADT therapy. However, whilst the supra-physiological testosterone values stimulates the T+ population leading to proliferation, it overstimulates the AR++ population, which results in cell death or reversion to the T+ phenotype [16]. Loss of AR++ cells results in decreased PSA response to testosterone injections 2 and 3. However, the slight

increase in PSA response on the 4th injection (compared to the 3rd injection) represents an increase in the T+ cells while the AR++ population was effectively eliminated by the first three testosterone doses.

2.2 | Mathematical Model

We have a long history of developing mathematical models to better understand, develop and predict cancer treatment strategies [17–20]. We develop a minimal model starting with the dynamics of T+ cells and represent them by resource-driven proliferation and death. The dynamics of T+ cells can be represented by resource-driven proliferation and death. Since they are sensitive to ADT, we refer to them as sensitive S cells:

$$\dot{S} = rS\left(\frac{U_S}{U_S + 1} - de^{-U_S}\right),$$

where $U_S = X_S T_E$, with X_S as a factor representing AR expression for the sensitive cells, and T_E representing testosterone normalized by the patient's baseline value T_0. The other parameters include r as the growth rate and d as the death rate relative to the growth rate. The dynamics of the AR++ cells can be represented similarly with also the addition of death caused by testosterone toxicity. We assume the AR++ cells will undergo apoptosis at supraphysiological testosterone concentrations. The resistant cells can be represented as:

$$\dot{R} = rR\left(\frac{U_R}{U_R + 1} - de^{-U_R} - \frac{d_{\text{tox}}}{1 + e^{-\alpha(T_E - \beta)}}\right).$$

Here, the toxicity term is represented with a sigmoidal shape to take effect somewhat sharply, only at high testosterone levels. The parameters include d_{tox} as the death rate due to testosterone toxicity, and α and β are factors that shape the sigmoid function.

Finally, we assume that mPC phenotypes produce PSA relative to testosterone metabolism. So, the PSA is calculated at each time point from the following equation:

$$PSA = r\left(S\frac{U_S}{U_S + 1} + R\frac{U_R}{U_R + 1}\right).$$

We fix all but four parameters and randomly sample from the initial fraction of resistant cells f_R, the AR expression of resistant cells X_R, the baseline testosterone T_0, and the death rate due to testosterone toxicity d_{tox}. We then compare the error from the model PSA output with the patient data and take the top 10 best-fit parameters sets. The output mean and standard deviation are shown in the PSA plot in Figure 3. The S and R subpopulations along with the total population are shown in Figure 3.

3 | Discussion

Although multiple new, life-prolonging therapies for metastatic prostate cancer have been introduced in the last two decades, deaths from prostate cancer continue to increase. Initial treatment of mPC with ADT is nearly always successful and tumor control is typically maintained for 2 to 4 years. However, evolution of resistance is virtually inevitable and transition to castrate resistant disease carries a grim prognosis. While several treatment agents are available for mCRPC, evolution of resistance is typically rapid, and average subsequent survival remains less than 2 years [3]. Thus, the proximate cause of death in most, perhaps all, patients with metastatic prostate cancer is evolution—that is, the Darwinian dynamics that allow prostate cancer cells to adapt to all currently available, and initially effective, treatments.

Evolution-based therapies typically change focus from killing as many cancer cells as possible to controlling the resistant population(s) that ultimately determine outcomes. Prior and ongoing studies have demonstrated that evolution-informed cycling of a residual treatment-sensitive population can suppress resistant cells to delay time to progression and increase overall survival. Such an approach has extended life in mCRPC [6]. The on/off treatment cycles in adaptive therapy superficially resemble intermittent therapy protocols [21]. However, the latter uses an induction period with prolonged continuous treatment at maximum tolerated dose (MTD). The induction period defeats the evolutionary strategy by greatly reducing or eliminating the treatment-sensitive population [15, 22]. This was observed in our patient who received 18 months of continuous ADT before the adaptive protocol. The tumor progressed after just three cycles.

For the current patient, in discussing treatment options for mCRPC, it became clear that most were not feasible. An attempt to add abiraterone to treatment regimen was unsuccessful because of intolerable side effects. The patient declined chemotherapy due to concern over exacerbating symptoms related to his familial neuropathy.

In the absence of other options, our multidisciplinary team relied heavily on prior clinical trials using BAT in which testosterone injections were used to treat patients with mPC [12, 23].

We hypothesized that sequential and strategically timed administration of testosterone could convert mCRPC to mCSPC through two mechanisms: (1) Promote T+ cells that require near physiological levels for proliferation and are sensitive to ADT; (2) Reduce the AR++ population which uses hyper-expression of androgen receptors, to proliferate in castrate levels of testosterone but undergo apoptosis via testosterone toxicity at supraphysiological levels.

Thus, we imposed iatrogenic evolutionary selection forces that increased fitness of the T+ phenotypes and decreased fitness of the AR++ phenotypes. This "directed evolution" achieved the desired goal of restoring the mCSPC state for, thus far, four successful adaptive cycles.

In summary, the patient's therapeutic regimen has two elements of evolutionary therapies. The first applies adaptive therapy by turning on and off ADT during periods when T+ cells seem to predominate. The second uses cycles of

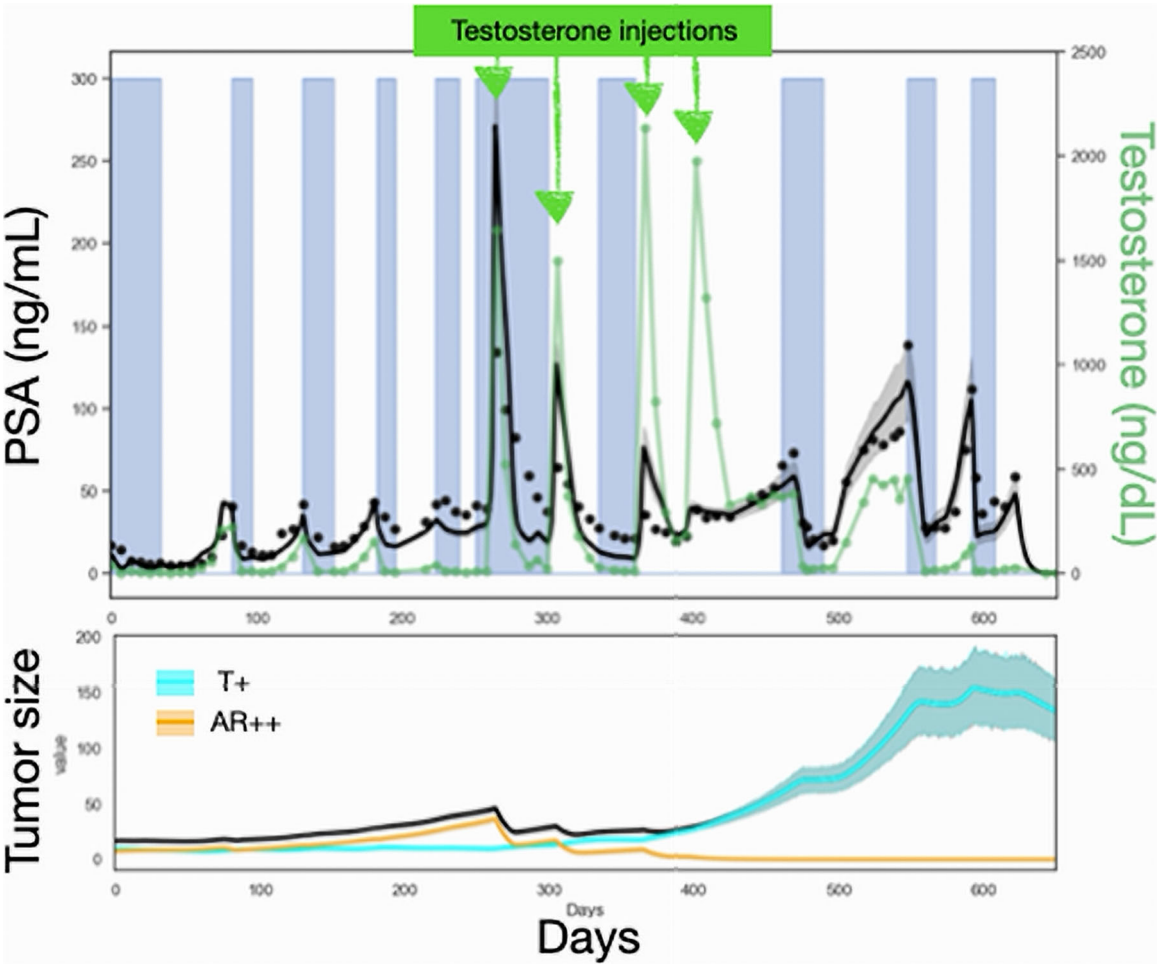

FIGURE 3 | The mathematical model uses the testosterone as input and is fit to the PSA values (upper). The T+ cells slightly dominate the population at the start, the AR + + cells take over during progression, and the T+ cells come back post testosterone injections whilst the AR + + cells die off (lower). The top 10 best-fits are shown with the mean and standard deviation of fR = 0.38 ± 0.1, XR = 75 ± 21, dtox = 0.13 ± 0.04 Day-1, and T0 = 164 ± 25 ng/dL. The constant parameters include $r = 0.015$ Day-1, d = 0.0053 Day-1, XS = 3, $\alpha = 3$, and $\beta = 1.5$. [Color figure can be viewed at wileyonlinelibrary.com]

testosterone injections as therapy when the AR + + cells predominate. As demonstrated by mathematical models, prostate cells adapt to ADT by increasing expression of androgen receptors forming a resistant (AR + +) population that proliferates in castrate levels of testosterone. In mCRPC, the AR + + population is dominant but vulnerable to the lethal effects of testosterone injection. Here, the T+ cells are the resistant population because they can proliferate in physiological or supraphysiologic testosterone levels.

In combination these two phases of adaptive therapy result in double-bind therapy [24]. ADT steers an evolutionary shift towards BAT efficacy, and BAT steers an evolutionary shift towards ADT efficacy. In effect, this strategy represents directed evolution that maintains a mPC subpopulation distribution that permits prolonged tumor control.

Acknowledgments

The authors thank Dr. Samuel Denmeade for providing advice based on his prior and ongoing BAT trials. This study was supported by NIH grant U54-CA274507.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All relevant data is included in the manuscript and figures. Additional information is available through inquiries to the corresponding author.

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