# The importance of cellular androgenic hormonal mechanisms in treatment and progression of prostate cancer

## Hücresel androjenik hormonal mekanizmaların prostat kanseri tedavisinde ve progresyonundaki önemi

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**ABSTRACT**

Since the seminal work of Charles Huggins, androgen deprivation therapy (ADT) has been the treatment of choice of advanced PCa. The dramatic effect of ADT results from the dependence of prostate cancer cell on androgen for growth and normal function. Most prostate cancer however are able to growth in absence of serum testosterone, a phenomenon coined castration-resistance. This largely explains why, despite initial response of great magnitude, ADT is essentially a palliative treatment with little if no benefit on survival. In the last 10 years, the basic mechanisms explaining resistance to castration have been unveiled leading to the development of new agents able to revert that mechanisms, including recently FDA approved abiraterone and enzalutamide. The most important adaptation progresses are the ability of the cell to produce its own androgens and the mutation and overexpression occurring at the level of the AR. These mechanisms and their practical implications are explained hereafter.

**Key words:** Prostate cancer, androgen receptor, cellular hormonal mechanisms, progression, treatment

**ÖZET**

Carles Huggins'in çığır açan çalışmaları sonucu ileri evre prostate kanseri standart tedavisi Androjen Baskılama Tedavisidir (ABT). ABT tedavisi prostat kanser hücrelerinin çoğalma ve normal fonksiyon görebilmesi için androjene bağımlı olması esasına dayanır. Ancak yine de pek çok prostat kanseri kastrasyona dirençli durum olarak tanımlanan testosteron olmadan da bağımsız olarak çoğalma ve büyüme gösterebilmektedir. Bu durum androjen baskılama tedavisinin her ne kadar ilk başta tedavi yanıtı çok iyi olsada palyatif bir tedavi olduğunu ve sağkalım üzerinde hiç yada çok az bir avantaj sağladığı gerçeğini açıklamaktadır. Son 10 yıl içinde kastrasyona direnç mekanizmalarının anlaşılmasıyla birlikte bu mekanizmaları tersine çevirecek örneğin FDA onaylı abiraterone ve enzalutamide gibi ilaçlar geliştirilmeye başlanmıştır. Bu gelişmeler içindeki en önemli nokta hücrenin kendi androjenini üretebilme yeteneği ve androjen reseptör düzeyinde gerçekleşen mutasyon ve overekspresyondur. Derlemede bu mekanizmalar ve bunların pratik sonuçları değerlendirilmektedir.

**Anahtar kelimeler:** Prostat kanseri, androjen reseptörü, hücresel hormonal mekanizmalar, progresyon, tedavi

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Prostate cancer (PCa) is the most frequent cancer in male, and one of the leading causes of death by cancer. Despite majors advances in screening, early detection and local treatment, a large number of patients will progress to an advance stage requiring systemic treatment. Since the seminal work of Charles Huggins, androgen deprivation therapy (ADT), by means of orchiectomy or administration of estrogens, has been the cornerstone treatment of advanced PCa (1). Very early, although, Huggins recognized that there were many failure to ADT: "*The first series of patients with prostatic cancer treated by orchiectomy comprised 21 patients with far advanced metastases; only 4 of them survived for more than 12 years. Despite regressions of great magnitude, it is obvious that there were many failures of endocrine therapy to control the disease but; on the whole, the life span had been extended by the novel treatments and there had been a decrease of man-pain hours*" (2). Indeed, despite inducing a regression of large magnitude, ADT is usually followed by a regrowth of the tumor, a mechanisms known as androgen independence (AIPC), hormone resistance (HRPC), or more recently castration resistance (CRPC).

Very early, two opposing theories have emerged to explain the progression in a low testosterone environment (3). The first theory, known as the *clonal selection* theory, postulates on the existence of pre-existing clones of CRPC cells that escape any hormonal manipulation at anytime. The second theory, known as the adaptive theory, postulates on the existence of subsequent phases of up and down regulation or mutations of important genes that help the cancer

cells survive and (re)grow after ADT. It is very likely that these two theories coexist in PCa patients. In most patients, PCa are heterogeneous polyclonal tumors made of different population that respond heterogeneously to ADT. For the physicians, however, the major developments have been done with the development of new drugs tackling the androgen receptor pathway. Therefor, we will review mainly the adaptative theory since it underlies the development of two drug with curative potential in CRPC, abiraterone and enzalutamide.

## What are the physiological bases of androgen dependence of normal and cancerous epithelial cells?

The prostate is a sexual accessory gland involved in the production of part of the semen. The growth and maturation of the normal prostate is dependent on the secretion of androgens by the testis.

The regulatory effect of androgens is mediated through the ***androgen receptor (AR)***, a member of the nuclear steroid hormone receptor super family. The AR gene is located on the X chromosome (Xq11–12). As the other nuclear steroid hormone receptors, it comprises 4 domains: a N-terminal domain (NTD), a highly conserved DNA-binding domain (DBD), a short hinge region and a moderately conserved C-terminal domain, the ligand binding domain (LBD) (4). Binding of ligand to the AR induces a conformational change allowing the release of the AR from its chaperone protein, change to a more active form, nuclear translocation, homodimer formation and DNA binding. The binding of the AR to AREs allows the recruitment of AR co-activators and/or co- repressors that regulate the transcriptional machinery (5).

The normal prostatic epithelium is made of different compartment, with different level of AR expression and therefore different sensitivities to androgens (Figure 1) (6). The basal compartment that is directly separated from the stromal compartment by the basal membrane comprises *prostate adult stem cells* (PAS) and *transit-amplifying* (TA) cell. PAS cells are present in very small proportions (<5%), have a very high self-renewal capacity and expresses no AR. Infrequently, PAS cells will differentiate into transit-amplifying (TA) cell, a progenitor that undergoes a limited number of proliferative replications before terminal differentiation. As for the PAS cells, TA cells do not express AR and are dependent for proliferation on growth factors produced by stromal cells. After a limited number of cell divisions, TA cells mature into *intermediate cells* that express AR mRNA, but not yet AR protein. AR protein will be found in intermediate cells migrating in the *luminal-secretory layer*. In these cells, AR activation promotes differentiation into secretory cells that will produce prostate-specific antigen (PSA) and other secretions product. Interestingly, in these luminal-secretory cells, AR activation also plays an anti-proliferative role, *e.g.* by up-regulating expression of cyclin-dependent kinase inhibitors p21 and p27(7).

*"Growth and maintenance of normal and cancerous prostate epithelial cell is dependent on the presence of androgen, mainly testosterone and its main intracellular derivative dihydrotestosterone."*

There has been an intense research to identify the specific epithelial cell subtype in which the initial carcinogenic process could initiate (8). In contrast to normal epithelial cells, AR expression is found in a wider variety of epithelial cancer cells. High Grade PIN and Prostatic Inflammatory Atrophy (PIA), two common PCa precursors, express high levels of AR (9). This suggests that early during prostatic carcinogenesis, there is a gain-of-function that converts the AR from a growth suppressor gene to an oncogene during prostatic carcinogenesis. A typical gain-of-function is the fusion by translocation of the promoter of the transmembrane protease serine 2 (TMPRSS2) gene, which contains androgen response elements (AREs), to selected members of the erythroblast transformation-specific (ETS) transcription factor gene family (10).

## The physiological basis of androgen deprivation therapy

In the normal prostate, the rate of prostatic cell proliferation is balanced by an equal rate of prostatic cell death such that neither involution nor overgrowth of the gland normally occurs with time. If an adult male is castrated, serum T rapidly decreases to below a critical value. As a result, the prostate rapidly involutes due to a major loss in the glandular epithelial cells that are androgen dependent and undergo apoptosis following castration. This mechanism relies on stromal-epithelial interaction since it requires paracrine activity of stromal AR, the apoptosis of glandular cells being initiated when besides DHT the level of growth factors decreases to a critical level (11). Several growth factors modulating DHT dependent survival have been identified, including the keratinocyte growth factor (KGF) and Transforming Growth Factor ß-1 (TGFß-1). Once the level of these growth factors decreases to below a critical level within a particular glandular cell, a major epigenetic reprogramming of this cell occurs, resulting in the activation of apoptosis (12).

Using the human PC-82 prostatic xenograft system, Kyprianou et al. demonstrated that ADT activates similarly the apoptotic pathway in androgen-dependent human PCa cells (13). Furthermore, they demonstrated that apoptosis of androgen-dependent human PCa cells induced by ADT does not require these cells to be in actively proliferating but that these cells die without leaving G0.

## The physiology of resistance to castration or how cells progress in a low testosterone environment?

It was clear from the seminal work of Huggins that despite clinical response of great magnitude the effect of ADT is of short duration and that the tumor rapidly regrowths in absence of circulating androgen. This phenomenon has been named over the years hormone-resistance, androgen-independence, although nowadays present term is resistance to castration (CRPC). In fact these terms overlap two clinical different scenarios. There are patients who primarily failed hormonal manipulations and progress shortly after initiation of ADT and other who will progress after a variable duration of ADT and yet, still remain sensitive to further hormonal manipulations.

There are two opposing theories explaining the emergence CRPC (Figure 1). The most widely accepted model is the "adaptation" model, which supposes that CRPC cells arise through genetic/epigenetic conversion of previously androgen-dependent cells during conditions of ADT. The alternative model is known as the "clonal selection", a model that suggests that emergence of CRPC reflects the proliferation of a previously quiescent population of rare castration-resistant cells within an otherwise androgen-dependent tumor (3).

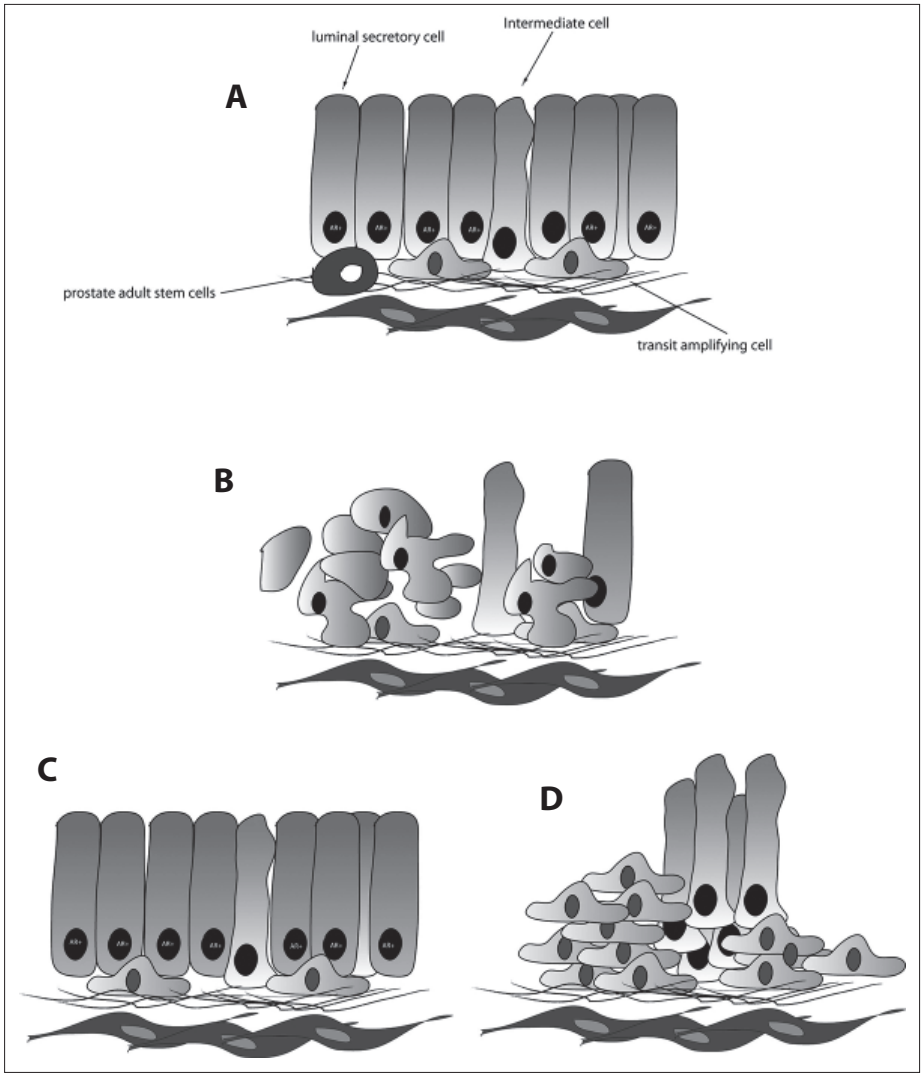


**Figure 1. A.** The prostatic epithelium originates from AR negative (AR-) prostate adult stem cells (PSA). These generate AR- transit amplifying (TA) cells and then AR- intermediate cells (I). AR positive luminal secretory cells are the final adults secreting prostate epithelial cells. **B.** Upon suppression of circulating androgen AR+ luminal cells massively induce apoptosis. **C.** The adaptative theory of castration resistance presuppose that some luminal secretory cells adapt their (epi) genetic program to allow growth in absence of androgens. **D.** The clonal theory of resistance to castration presuppose that the cancer develop from a pool of AR- cells that is already present in the prostate.

**Table 1.** Historical second-line hormonal manipulations used in CRPC patients (adapted from Tombal et al.

| *Drug* | *Patients (n) per trial* | *Number of trials* | *% PSA Response (range)* | *Duration (months)* |
|---|---|---|---|---|
| Bicalutamide (150 mg qd ) | 31-52 | 4 | 14 - 45 | 4 |
| Flutamide (250 mg tid) | 101 | 1 | 23 | 4,2 |
| Nilutamide (200 or 300 mg qd) | 14-28 | 2 | 29-50 | 7-11 |
| Ketoconazole (200- 400 mg tid) + hydrocortisone ± AAW | 28-128 | 6 | 27-63 | 3.5 -20 |
| DES (1-3 mg) | 21-42 | 2 | 24-43 | NA-2.8 |
| Prednisone | 29-101 | 4 | 22-34 | 2-4.2 |
| Hydrocortisone | 30-230 | 3 | 14-20 | 2.3-4 |
| Dexamethasone | 19-38 | 3 | 28-61 | NA |

**NA:** non available

## The adaption theory and the role of the AR in resistance to castration

The major argument in favor of the "adaptation" model is the evidence of retention of AR signaling in CRPC. Even when PCa progresses to CRPC, AR activation and signaling remains sustained through a variety of mechanisms (14-16). Notably, castration-resistant tumors express AR as well as AR target genes such as PSA, indicating that pathway activity is intact (17).

Physicians knew this since many years. Indeed, in absence of curative second line such as docetaxel or abiraterone, most physicians have historically prescribed antiandrogens, adrenal synthesis inhibitors, estrogens and derivative, or steroids. Most of these agents, because they interfere with the AR induced transient decrease of PSA, sometimes for a few months (see Table 1 and Tombal et al. (18) for detailed descriptions). Such treatment however were based exclusively the results of phase II trial reporting modest PSA decrease for short duration. There is no reported phase III trial. Objective responses are seldom reported and, so far, in contrast with the novel agents abiraterone and MDV3100, there has never been published reports of OS benefit.

In the last few years, however there has been a more intense scientific research leading to the unveiling of several mechanisms explaining the of an AR dependent pathway (see Figure 2).

### *Increased production of AR*

Normal prostate epithelial cell normally possess one copy of the AR gene. Amplification of AR gene copy number occurs seldom in primary PCa but is found in approximately one-third of CRPC (19-21). This results in a higher quantity of the wild-type AR protein and therefore in a higher sensitivity and responsiveness to low level of circulating or intracellular androgens, causing a survival and growth advantage upon castration. Palmberg et al. have demonstrated that patients with an AR gene amplification progressing after a first line of LHRH agonist had a 4.569 higher likelihood to respond to a subsequent maximal androgen blockade (22). In addition, an increased AR level can convert antiandrogens such as bicalutamide, from an AR antagonist to an AR agonist, being one of the explanation of the well-known antiandrogen withdrawal response (23). Increased levels of

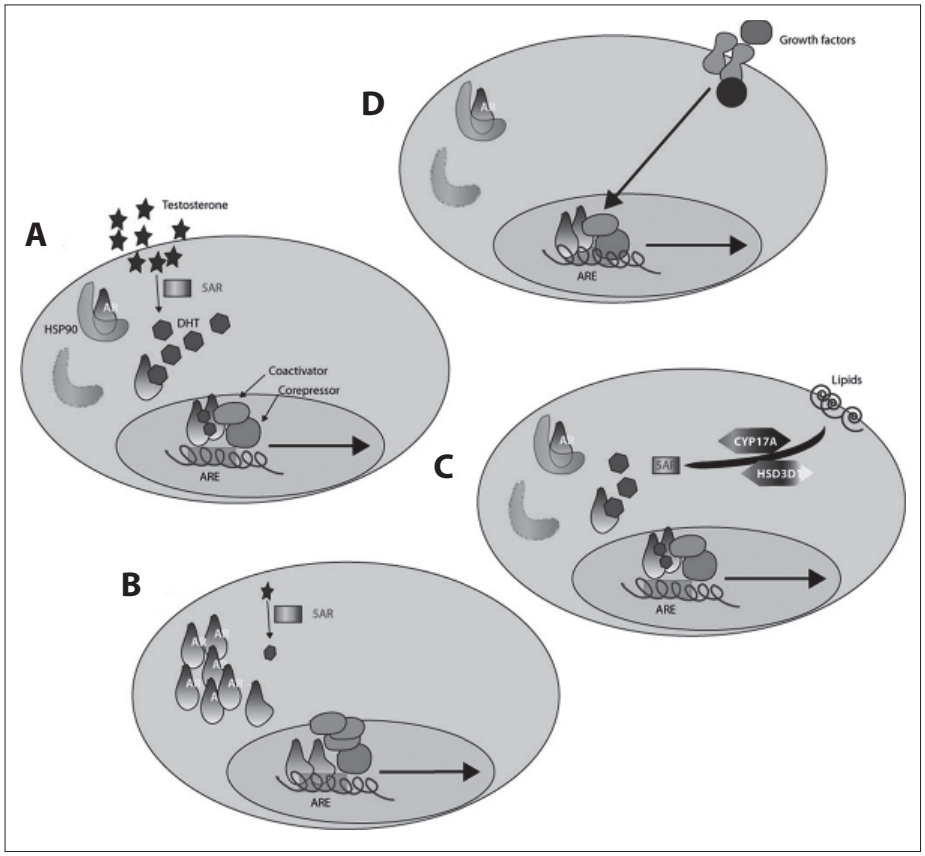


**Figure 2**. **A.** Serum testosterone (T) freely diffuse in the prostatic cells were it is rapidly converted in more active dihydrotestosterone (DHT). DHT binds to the androgen receptor (AR) and induces release from chaperone protein HSP90, conformational change, homodimerization and translocation to the nucleus where it binds to the ARE of the DNA to stimulate transcription. This is facilitated by recruitment of co-activators and co-repressors. **B, C,** and **D** represent three mode of AR dependent growth in low testosterone environment. **A.** Increase number of AR copies or mutation of the AR leading to auto-activated AR. **B.** Intracellular production of androgens through overexpression of enzymes from the steroidogenesis pathways. **D.** Direct growth-factor androgen ligand activation or the AR.

AR protein result primarily from AR gene amplification, but also from increased transcription rates, or stabilization of the mRNA or protein (24, 25). These observations have motivated the search for second-generation anti-androgens with greater affinity, using cells that overexpress AR (26). Enzalutamide (formerly known as MDV3100) is an androgen-receptor–signaling inhibitor chosen for clinical development on the basis of activity in PCa models with AR overexpression. Enzalutamide is distinct from the currently available antiandrogen agents, such as bicalutamide, in that it inhibits nuclear translocation of the androgen receptor, DNA binding, and coactivator recruitment (26). A, double-blind, placebo-controlled phase III trial, conducted in 1199 CRPC progressing after chemotherapy has demonstrated the benefit of enzalutamide (27). The median overall survival was 18.4 months (95% confidence interval [CI], 17.3 to not yet reached) in the enzalutamide group versus 13.6 months (95% CI, 11.3 to 15.8) in the placebo group (hazard ratio for death in the enzalutamide group, 0.63; 95% CI, 0.53 to 0.75; P<0.001). The superiority of enzalutamide over placebo was also shown with respect to reduction in the prostate-specific antigen (PSA) level by 50% or more, the soft-tissue response, the quality-of-life response rate, the time to PSA progression, and the radiographic progression-free survival

*"Upon suppression of androgens, normal and cancerous epithelial cells induce a massive cell death process, this is the base of androgen deprivation therapy."*

### *Gain-of-function mutations of AR*

Several mutations have been identified that may confer increased AR protein stability, greater sensitivity to androgens, aberrant responses to antiandrogens or other steroid

*"In a low androgen environment, cells will progress into a "castration resistant" stage."*

hormones, ligand-independent activity, or increased recruitment of AR co-activator proteins (28-32). Because AR mutations occur in stochastic and heterogeneous manner in different metastases it is extremely difficult to have a correct estimate of the real frequency of AR mutations in patients. An updated and exhaustive list of AR gene mutations can be found in the McGill Androgen Receptor Gene Mutation Database*. AR gene mutations in the LBD may alter ligand binding, leading to AR activation by AR antagonists and other ligands that do not activate wild-type AR, another explanation to the anti-androgen withdrawal syndrome.

In rare cases, mutated AR may lack the entire LBD leading to an auto-activated mutant that mediates AR signaling independent of any ligands (33). Such AR splicing variants lacking part of or the entire LBD, *ARΔLBD*, have been generated artificially in vitro to demonstrate that the AR can be constitutively active (34). These *ARΔLBD* are sufficient to confer ligand-independent and castration-resistant growth. The clinical relevance of these truncated AR variants has been characterized by developing specific technologies (35). In 2009, Hu et al. have uncovered seven AR variant lacking the LBD due to splicing of "intronic" cryptic exons to the upstream exons encoding the AR DNA-binding domain (35). Two of these *ARΔLBD* showed an average 20-fold higher expression in CRPC compared to hormone-naive PCa. More recently, Watson PA et al. demonstrated that *ARΔLBD* increase acutely in response to ADT, are suppressed by testosterone, and, in some models, are coupled to full-length AR mRNA production (36). Noteworthy, Watson et al. also showed that anti-androgens, such as MDV3100, or selective siRNA silencing of full length AR, block the growth of PCa cells, suggesting that the growth-promoting effects of *ARΔLBD* are mediated through full length AR. These authors hypothesize that the increase in *ARΔLBD* expression in CRPC is an acute response to castration rather than clonal expansion of cells expressing *ARΔLBD*. More

* http://androgendb.mcgill.ca/

*"Castration resistance is mainly driven by overexpression or mutation of AR or direct production of androgens by the adrenals or prostate cancer cells."*

recently, Hörnberg et al. have demonstrated that *ARΔLBD* expression was increased in CRPC compared to hormone naïve bone metastases and associated with a particularly poor prognosis (37).

*Intracellular steroidogenesis*

One of the most interesting observation from the last five years is the ability of CRPC to synthetize androgen *de novo* or convert adrenal androgens into T and DHT by expressing or up-regulating steroidogenic enzymes including CYP17A1, HSD3B1, HSD17B3, CYP19A1, or UGT2B17 (38-41). Already in 1993, F. Labrie, the father of maximal androgen blockade, suggested that adrenal androgens could induce AR signaling after intra-prostatic conversion despite low levels of circulating testosterone (42). In addition, several studies had shown already that the presence of a residual production of testosterone and/or its precursor was predictive on the response of second-line antiandrogens. Noteworthy, the benefit of adding an antiandrogen to a GnRH agonist seems primarily linked to the ability of that GnRH agonist to effectively suppress testosterone and to the importance of androgens secretion by the adrenals. For example, Narimoto et al. have examined the effects of flutamide as a second-line antiandrogen in 16 CRPC patients initially treated with bicalutamide (43). A PSA decline ≥ 50% was observed in 50% of the patients with a median response of 6.25 months. Elevated baseline androstenediol level was a predictive factor of PSA response and a low DHEA group a predictor of a prolonged response

In 2005, Titus et al. measured tissue level of T and DHT from 18 men with local recurrence during ADT and 18 men with benign prostatic hyperplasia (BPH) receiving no hormonal treatments (41). T levels were similar in CRPC (3.75 pM/g tissue) and BPH (2.75 pM/g tissue, Wilcoxon two-sided, P=0.30). DHT levels decreased by 91% in CRPC (1.25 pM/g tissue) compared with BPH (13.7 pM/g tissue; Wilcoxon two-sided, P < 0.0001) although DHT levels in most specimens of recurrent CRPC were sufficient for AR activation. His conclusions supported the Labries's hypothesis of an intracellular conversion of adrenal androgens to T and DHT. It is however Montgomery and coworkers that demonstrated that in addition to converting adrenal androgens, CRPC cells were also capable of synthetizing *de novo* androgens from membranes' cholesterol molecules. They showed that median T levels within metastases from CRPC men are approximately threefold higher than levels within the primary untreated PCa (39). They also elucidated the mechanism by demonstrating that this increased production of androgens from intracellular precursors was caused by up-regulating the expression of steroidogenic enzymes FASN, CYP17A1, HSD3B1, HSD17B3, CYP19A1, and UGT2B17 and the down-regulating SRD5A2 expression (P < 0.001 for all)(39).

Abiraterone acetate is a selective inhibitor of androgen biosynthesis that potently and irreversibly blocks CYP17, a crucial enzyme in testosterone and estrogen synthesis, resulting in virtually undetectable serum and intratumoral androgens and antitumour activity in both chemotherapy-naive and chemotherapy-treated patients with metastatic CRPC cancer (44, 45). Abiraterone combined with prednisone has been compared to prednisone alone 1195 metastatic CRPC who had previously received docetaxel (46). After a median follow-up of 12.8 months, overall survival was longer in

*"Enzalutamide (formely MDV3100) and abiraterone, by specifically targeting these pathways, can extend overall survival."*

the abiraterone acetate-prednisone group than in the placebo-prednisone group (14.8 months vs. 10.9 months; hazard ratio, 0.65; 95% confidence interval, 0.54 to 0.77; P<0.001). All secondary end points, including time to PSA progression, progression-free survival, and PSA response rate, favored abiraterone.

### Ligand-independent activation of AR

The activation of different signal transduction pathways in CRPC cells can enhance the activity of the AR or its co-activators in the presence of low levels or even in the absence of androgens. For example For example, IL-6, KGF, EGF and IGF-1 are overexpressed in CRPC and can stimulate AR transcription in the absence of ligand (47, 48). The MAPK and PI3K/AKT pathways are probably the leading pathways, which regulate the phosphorylation of AR coactivators, such as SRC-1 and TIF2, or the AR protein itself (47-49).

## Conclusions

As long as primary ADT remains the main therapeutic for advanced PCa, CRPC will remain the main challenge to be tackle by physicians. Extensive investment in basic sciences has started to unveil some of the primary mechanisms underlying CRPC acquisition. Already, it appears that it is very unlikely that a unique prevailing pathway will emerge. To treat that disease, we will need to solve the individual heterogeneity and develop test to allow individualizing treatment to specific situation.

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