

# Paradigm Shift in the Concept of Hormonal Milieu of Prostate Cancer

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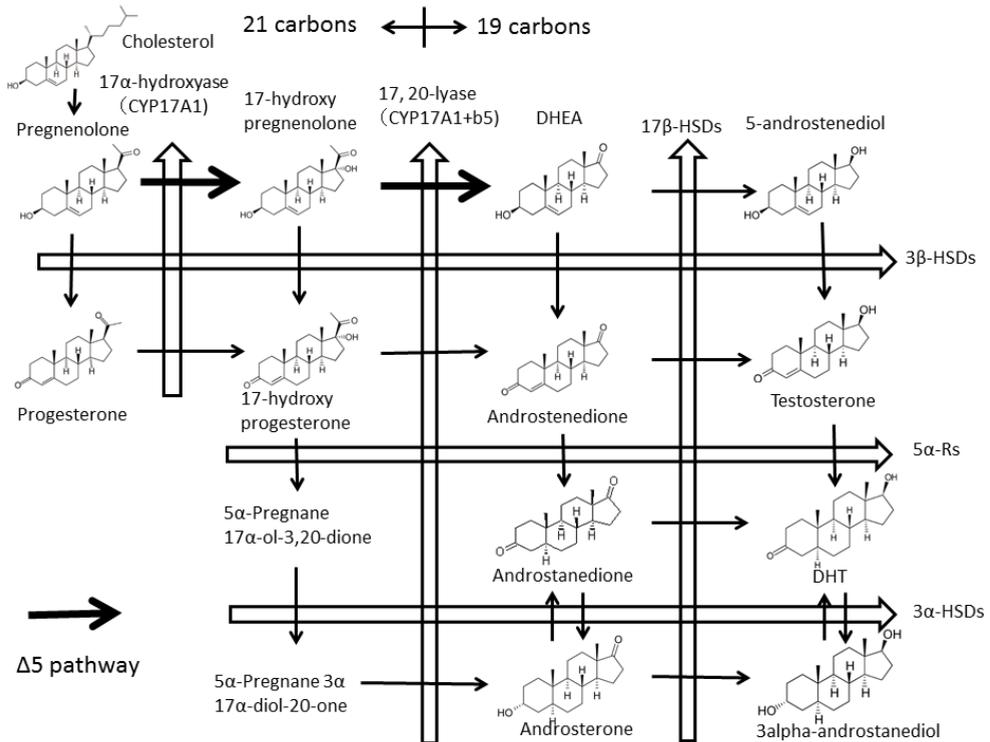
## 1. Introduction

Androgens and the androgen receptor have been implicated in a number of human diseases and conditions, most notably prostate cancer. [1] Huggins and Hodges in 1941 demonstrated that bilateral orchiectomy or estrogen treatment is an effective treatment for prostate cancer.[2] Based upon their findings, androgen deprivation therapy (ADT) has remained the main therapeutic option for patients with advanced prostate cancer for about 70 years. Different therapeutic approaches, including surgical or medical castration with gonadotropin-releasing hormone (GnRH) agonists, and an antiandrogen therapy, have become the gold standard for prostate cancer treatment with metastases. ADTs are quite successful, leading to regression of tumors in a majority of cases; however, 12-18 months later, most prostate cancers recur in a more aggressive, castration-resistant form (castration-resistant prostate cancer (CRPC)) that is incurable. Median survival for these patients after recurrence is 24-36 months.[3, 4] New ADTs and other promising therapies for CRPC have been actively developed.[5] This review will provide an overview of prostate cancer aggressiveness and the androgen levels in blood and prostatic tissues, androgen milieu during ADT, androgen milieu during CRPC, and the prospects of ADT on the ground of the androgen milieu in the prostate.

## 2. Androgen milieu in blood

Biosynthetic pathway for androgen synthesis is the  $\Delta$ -5 pathway in primates like humans. [6] (Fig. 1) As a result, a large amount of dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEA-S) are in the blood in humans. In rodents, biosynthetic pathway for androgen synthesis is the  $\Delta$ -4 pathway. In human males, testosterone is the major circulating androgen. More than 95% of testosterone is secreted by the Leydig cells, situated in the testicular interstitium, while the adrenal cortex also contributes to this production. [7, 8] The Leydig cells produce testosterone in response to hormonal stimulation by the pituitary gonadotropin luteinizing hormone (LH). Testosterone plays a key role in health and well-being as well as in sexual functioning. Testosterone effects can be classified as virilizing and anabolic effects including growth of muscle mass and strength, increased bone density and strength. Serum testosterone levels decline with advancing age. [9] Recently, a pooled analysis of moderately sized prospective studies

including 3886 cases of prostate cancer and 6438 matched controls reported no association of circulating androgens and risk of prostate cancer, either overall or for subgroups according to tumor stage or grade. [10] Prostate cancer risk and tumour aggressiveness are not related to serum levels of total and free testosterone. [11] However, this negative result does not deny the role of androgens for the prostate cancer, rather emphasizes the importance of the androgen metabolism in the prostate. It is not clear to what extent the testosterone and dihydrotestosterone (DHT) in the prostate derive from adrenal androgens or other steroid precursors.



\*b5: cytochrome-b5 reductase

CYP17A1: Cytochrome P450, family 17, subfamily A, polypeptide 1

DHEA: dehydroepiandrosterone

DHT: dihydrotestosterone

HSD: hydroxysteroid dehydrogenase

5 $\alpha$ -Rs: 5 $\alpha$ -reductases

Fig. 1. The androgen metabolic pathway and enzyme related products of human being. Biosynthetic pathway for androgen synthesis is the  $\Delta^5$  pathway in primates like humans. As a result, a large amount of DHEA and DHEA-S is in the serum in humans.

### 3. Androgen milieu in the prostate

Testosterone and DHT can be synthesized from DHEA with various androgen synthetic enzymes in the human prostate. The conversion of testosterone from 17-ketosteroid, androstenedione, is said to be catalyzed by the 17 $\beta$ -hydroxysteroid dehydrogenase (HSD) type 1, 3, 5, 12. [12] 17 $\beta$ -HSD type3 is mainly expressed to convert into testosterone from androstenedione in the testis. Aldo-keto reductase family 1, member C3 (AKR1C3) , known as 17 $\beta$ -HSD type5, is mainly expressed to convert into testosterone from androstenedione in the prostate. [13] Humans have three isoenzymes of 5 $\alpha$ -reductase. Both type 1 and type 2 5 $\alpha$ -reductase catalyze the conversion of testosterone to DHT. [14, 15] Type 1 enzyme (encoded by SRD5A1) is expressed mostly in skin and hair, while type 2 enzyme (encoded by SRD5A2) is located primarily in androgen target tissues, including genital skin and prostate. Recently, the existence of 5 $\alpha$ -reductase type 3 (encoded by SRD5A3) is identified. [16] Godoy et al. showed that expression of 5 $\alpha$ -reductase type 3 in "classical" as well as "non-classical" androgen-regulated tissues is consistent with 5 $\alpha$ -reductase type 3 enzyme having functions other than converting testosterone to DHT in human tissues, such as participation in the N-glycosylation process. [17] They also showed that over-expression of 5 $\alpha$ -reductase type 3 in breast, testis, lung, thyroid, and particularly prostate cancer, compared to their benign counterparts, suggests a potential role for 5 $\alpha$ -reductase type 3 as a biomarker of malignancy, and over-expression of 5 $\alpha$ -reductase type 3 in androgen sensitive prostate cancer and CRPC suggests a potential role for this enzyme in synthesizing DHT in both an androgen-stimulated and an androgen-deprived human prostate microenvironment. Therefore, the prostate is not only the androgen dependent organ but also the androgen productive organ. Recently, DHT mainly synthesizes via testosterone as stated above, but also synthesizes without testosterone biosynthesis, 'backdoor' pathway. [14, 18, 19] An alternate 'backdoor' pathway to DHT was elucidated in the tammar wallaby pouch young, and studies in knockout mice showed that this pathway uses 5 $\alpha$ -reductase type 1 to convert 17-hydroxyprogesterone to 5 $\alpha$ -reduced androgen precursors.[20] The role of 'backdoor' pathway to DHT in human androgen metabolism, however, is not clear yet. The traditional belief that prostate cancer growth is dependent on the serum testosterone level has been challenged by recent negative studies in non-castrated men.[21, 22] The evidence clearly indicates that there is a limit to the ability of androgens to stimulate prostate cancer growth. A Saturation Model based on androgen-AR binding provides a satisfactory conceptual framework to account for the dramatic effects seen with castration as well as the minor impact of testosterone administration in non-castrated men.[23]

### 4. Prostate cancer aggressiveness and the androgen milieu in the prostate and in blood

Sex hormones in serum have been hypothesized to influence the risk of prostate cancer. Large prospective study and a pooled analysis of moderately sized prospective studies did not show convincing evidence of a relationship between serum sex hormones and prostate cancer. [10, 24] However, these negative results do not deny the role of androgens for the prostate cancer, rather emphasizes the importance of the androgen metabolism in the prostate. It is not clear to what extent the testosterone and DHT in the prostate derive from adrenal androgens or other steroid precursors. The 90-95% decrease in serum testosterone is

observed after castration; however, the prostate efficiently transforms the adrenal androgen precursors DHEA-S, DHEA, and androstenedione into the active androgens, testosterone and DHT. All the enzymes are reported to exist in the prostate metabolizing from androgen precursors to testosterone and DHT. [25] The plasma concentration of DHEA-S is 100-500 times higher than that of testosterone. [26] High circulating levels of DHEA-S provide large amounts of the precursors required for conversion into active androgens in the prostate. The active androgens made locally in the prostate act in the prostate. In our data, the level of DHT in the prostate before ADT was not correlated with the serum level of testosterone, but was correlated with the serum level of DHEA and DHEA-S. [26] These results suggested that DHT in the prostate comes not only from testosterone produced in the testes, but also from adrenal androgens. There are still many questions about the androgen environment in the prostate in patients with prostate cancer. There have been few reports about the relations between histological aggressiveness and the DHT levels in the prostate in patients with prostate cancer. We revealed that the DHT levels in the prostate in patients with Gleason scores of 7 to 10 were significantly lower than those with Gleason scores of 6 and under, although there were no significant differences between the patients with Gleason scores of 7 to 10 and the patients with Gleason scores of 6 and under prostate cancer with respect to serum levels of androgens. [27] The result of the association between the DHT level in the prostate and the Gleason score gives useful information to predict the biological aggressiveness of prostate cancer and consider the treatment of the patients with such cancers. One hypothesis as to the correlation between clinical aggressiveness and low androgen levels in the prostate is that the hormonal milieu with low androgen levels might be varied enough to disrupt the normal growth and maintenance of the prostate tissue, and may cause the resultant compensatory hyperplasia that increases the risk of a consequent selection of androgen independent, aggressive prostate cancer formation due to low androgen levels. [28] Several studies suggested that men with smaller prostates had more high-grade cancers and more advanced diseases. [29-31] Prostate size was inversely associated with the risk of high grade cancer. The Prostate Cancer Prevention Trial (PCPT), a Phase III, randomized, double-blind, placebo-controlled trial of finasteride, a 5- $\alpha$ -reductase inhibitor, for the prevention of carcinoma of the prostate was held. [32] A total of 18,882 essentially healthy men, aged 55 and older, had been randomized to receive finasteride (5 mg daily) or placebo for seven years. PCPT results suggested that finasteride could reduce the frequency of prostate cancer by 25% compared with placebo. However, the patients with Gleason scores of 7 to 10 were detected significantly more frequently in the finasteride group than in the placebo group. Several questions were raised by the results of the trial that finasteride could induce the development of high grade disease. They did not find evidence that the drug caused changes in tumor composition that might contribute to aggressive cancer, though they don't entirely rule out the possibility that finasteride may have led to high-grade prostate cancer in some men in the study. One possible explanation was the occurrence of over-grading due to histological alterations. Several reports, however, showed that although finasteride use might cause some alterations in the assignment of the Gleason grade, a consistent hormonal therapy effect with finasteride treatment has not been observed. [33] Lucia et al. revealed that finasteride may have contributed to the increased rate of high-grade cancers detected in the PCPT by making the prostate smaller, helping the biopsy find the cancer. [34] Moreover, the results of the Reduction by Dutasteride of

Prostate Cancer Events (REDUCE) trial using the dual 5- $\alpha$ -reductase isoenzyme inhibitor dutasteride, has recently been reported.[35] The REDUCE trial, contrary to the Prostate Cancer Prevention Trial (PCPT), is performed in 8,231 men ages 50-75, who would have been candidates for biopsy anyway because of PSA values of 2.5 - 10 ng/ml. Although over the course of 4 years, significantly fewer prostate cancers were detected in men taking dutasteride than in those taking placebo, representing a relative risk reduction of 22.8% ( $P < 0.001$ ), there were 12 tumors with a Gleason score of 8 to 10 in the dutasteride group, as compared with only 1 in the placebo group ( $P = 0.003$ ). These findings and our results suggest that biologically aggressive prostate cancer can exist under a low DHT environment, and may proliferate under the low DHT environment with finasteride or dutasteride medication where the prostate cancer of a low malignancy with high DHT dependency cannot easily exist. High levels of androgen receptor (AR) are said to be associated with the increased proliferation, markers of aggressive disease, and to be predictive of decreased biochemical recurrence-free survival independently. [36] This confirms the role of AR in tumor growth and progression in hormonally naive prostate cancer. Several reports and our results revealed that 17 $\beta$ -HSD5 (known as AKR1C3) positive cases were significantly associated with clinical stage. [37, 38] Fung et al. revealed that elevated expression of AKR1C3 is highly associated with prostate cancer. [37] We also revealed that both AR and androgen-converting enzymes were up-regulated in the high-grade or advanced prostate cancer. [38] AKR1C3 (17 $\beta$ -HSD5) may be involved in increasing the local concentration of testosterone in prostate cancer tissues, resulting in the progression, invasion and further development of prostate cancer following ADT.

## 5. Intraprostatic androgen milieu during androgen deprivation therapy

The castration level of serum testosterone is disputed; however, historically, circulating testosterone levels have been used to assess the efficacy of androgen depletion with a target total testosterone level below 50 ng/dl ( $< 1.74$  nmol/l). [39-42] In men with prostate cancer, orchiectomy reduces serum testosterone to anorchid levels within 12 hours. The clinical significance of different serum levels of testosterone yielded during ADT has not yet been well elucidated. The serum testosterone level in men being treated with ADT has never been categorically established. [43] The typical serum testosterone level in men after an orchiectomy is 14 ng/dl, which is equivalent to 0.5 nmol/l. Over the years, there have been many specialists who have argued that the appropriate serum testosterone level for a man who is being well-controlled on ADT should, in fact, be  $< 20$  ng/dl (0.7 nmol/l). Morote et al. have shown that 25% of men receiving continuous ADT with a GnRH agonist have a serum testosterone level  $> 50$  ng/dl. [44] Forty-four percent of the same group of men studied had a serum testosterone level that was consistently  $< 20$  ng/dl. Also, in the same group of men, they demonstrated a direct correlation between serum testosterone levels and time to development of CRPC. Perachino et al. have also reported a significant association between serum testosterone levels 6 months after initiation of ADT and overall survival. [45] Several reports showed that DHT in the prostate after ADT is supplied by the precursors of androgens from the adrenal origin. We showed that after ADT with castration and flutamide, the DHT level in the prostatic tissue remained at approximately 25% of the amount measured before ADT in the same patients. [26] Previous reports revealed that the mean DHT levels in the prostate tissue treated with ADT were between 10% and 40% of

those of untreated. [26, 46-50] Testosterone is converted to DHT by 5- $\alpha$  reductase in the prostate. Labrie et al. showed that even if testosterone levels in blood drop to low levels by castration, DHT levels in the prostate were maintained about 40% before the treatment. [47] These results showed that the considerable levels of DHT exist in the prostate after castration. We also revealed that the levels of DHT in the prostate before ADT were correlated with the serum levels of adrenal androgens. [26] We could not recognize the correlation between testosterone levels in blood and DHT levels in the prostate before the treatment; however, we recognized the correlation between DHT levels in the prostate and DHEA levels (or DHEA-S levels) in blood. The levels of DHT in the prostate after ADT using a GnRH agonist and flutamide were also correlated with the serum level of testosterone. The serum DHT levels after ADT were correlated with serum levels of adrenal androgen. The testosterone levels in blood after ADT are supplied from the adrenal androgen precursors converted in the prostate, and the adrenal androgen precursors are converted into DHT in the peripheral organs including the prostate. These results showed that DHT in the prostate can be made not only from testosterone derived from the testis but also from the adrenal precursors. These findings suggest that serum testosterone after ADT mostly comes from adrenal androgens converted in the prostatic cells. These results revealed that the prostate is the main DHT producing organ and DHT levels in the prostate are correlated with the adrenal androgen precursor levels and testosterone levels in the prostate.

Substantial levels of DHT remain in the prostate after castration; however, the influence on the change in the DHT levels in the prostate before and after ADT in connection with prostate cancer aggressiveness has not yet been elucidated. We revealed that the change in the DHT levels before and after ADT in the prostate with the prostate cancer of Gleason scores of 7 to 10 was significantly smaller than that in the prostate with the prostate cancer of Gleason scores of 6 or less.[51] Recently, 5- $\alpha$ -reductase type 1(SRD5A1) is reported to express in aggressive and recurrent prostate cancer. [38,52, 53] Arai et al. showed that the levels of adrenal androgens in prostate cancer tissues after ADT were similar to those in untreated prostate cancer. [54] Especially, DHEA was the most existing androgen precursor in prostate cancer tissues after ADT. The levels of DHEA were high in prostate cancer tissues, irrespective of ADT. They assumed that DHEA played a significant role in the synthesis of testosterone and DHT in prostate cancer tissues after ADT. Several reports revealed that AR signaling pathway is an important mechanism in proliferation of aggressive prostate cancer during ADT.[55] Stanbrough et al. revealed that AR transcriptional activity is reactivated in androgen-independent prostate cancer and identifies the intracellular conversion of adrenal androgens to testosterone as a mechanism mediating this reactivation. [56] These findings and our results suggest that the low DHT levels in the prostate with aggressive prostate cancers are probably sufficient to propagate the growth of the tumor, and the prostate with aggressive prostate cancer can produce androgens from the adrenal precursors more autonomously than that with non-aggressive prostate cancer does under the low testosterone environment with testicular suppression. Mostaghel et al. showed that the reduction of circulating androgens to castrate levels and interruption of AR signaling by androgen deprivation are neither reliably effective nor uniform in suppressing prostatic androgen-regulated gene and protein expression.[57] Their findings suggest that medical castration based on serum testosterone levels cannot be equated with ablation of androgen levels or androgen-mediated activity in the prostate

tissue microenvironment. These findings underscore the need for demonstrating suppression at the target tissue and molecular level before drawing conclusions regarding the clinical efficacy of hormonal treatment strategies. We showed that the serum PSA levels well reflect the androgen milieu in localized prostate cancer patients receiving ADT, which can be explained by the Saturation Model and disease control. [58] The androgen milieu in men with high Gleason score prostate cancer is probably less affected by conventional ADT than that in men with low score cancer, which was suggested to be associated with adrenal androgen levels. We also showed that in patients treated with ADT the pituitary-adrenal axis mediated by adrenocorticotrophic hormone has a central role in the regulation of androgen synthesis. [59] Serum adrenocorticotrophic hormone and adrenal androgen concentrations were correlated with the post-treatment PSA. Adrenocorticotrophic hormone mediated androgen synthesis is a potential target for advanced androgen deprivation therapy.

## **6. Androgen milieu in the castration-resistant prostate cancer**

The pathogenesis of CRPC involves a mixture of multiple pathways that increase and broaden the function of the AR, and others that bypass the AR.[60] Analysis of tumor samples from patients with CRPC has revealed several mechanisms utilized by tumor cells to reactivate the AR signaling at sub-physiological serum concentration of androgens or even in the presence of AR antagonists.[61] CRPC has been used synonymously with androgen-independent prostate cancer and hormone-refractory prostate cancer but is the preferred term, as we now know that many men with CRPC respond to additional manipulations that ablate or block prostate cancer growth stimulation by androgens. CRPC is clinically detected by a rise in PSA, typically defined as three consecutive rises over nadir in the context of castrate levels of serum testosterone and after anti-androgen withdrawal for at least 4 weeks and despite secondary hormonal manipulations and/or radiologic progression. In the face of testicular androgen suppression the adrenal androgens DHEA, DHEA-S and androstenedione retain the potential to stimulate prostate cancer growth since these steroids are converted to testosterone and DHT in the prostate, which are active androgens even after prostate cancer acquires the ability to activate during ADT. Chen et al. showed that a modest increase in AR mRNA was the only change consistently associated with the development of resistance to anti-androgen therapy.[62] This increase in AR mRNA and protein was both necessary and sufficient to convert prostate cancer growth from a hormone-sensitive to a hormone-refractory stage, and was dependent on a functional ligand-binding domain. Conventional AR antagonists showed agonistic activity in cells with increased AR levels; this antagonist-agonist conversion was associated with alterations in the recruitment of coactivators and corepressors to the promoters of AR target genes. Increased levels of AR confer resistance to anti-androgens by amplifying signal output from low levels of residual ligands, and by altering the normal response to antagonists. Mohler et al. revealed that recurrent prostate cancer may develop the capacity to biosynthesize testicular androgens from adrenal androgens or cholesterol. [63, 64] They showed that tissue levels of testosterone were similar in recurrent prostate cancer and benign prostate. They also showed that the DHT level in recurrent prostate cancer tissue was decreased to 18% of the level in benign prostate tissue. Testosterone and dihydrotestosterone occur in recurrent prostate cancer tissue at levels sufficient to activate AR. Montgomery et al. showed that soft tissue metastases in patients with anorchid serum testosterone contain levels of testosterone that are up to three times higher than those in prostate tumors in eugonadal men.[65]

Transcript levels of enzymes involved in androgen synthesis were up-regulated in the same tumors, suggesting that tumoral synthesis of androgens from cholesterol might occur.[66] Androgen-independent prostate cancer cell lines synthesize testosterone from radio-labeled cholesterol *in vitro*, and human prostate cancer xenografts are capable of synthesizing DHT from acetate and cholesterol, confirming that tumoral androgen synthesis is possible.[67] Thus, experimental and clinical evidence suggests that the enzymes of steroid metabolism are activated in the prostate cancer with CRPC state, and might provide multiple new targets for therapeutic intervention. Moreover, substantial evidences suggest that CRPC may not, in fact, be androgen independent, but occurs in a setting of continued AR-mediated signaling that is driven by the presence of residual tissue androgens. We used androgen independent and androgen sensitive cells, LNCaP cells, and examined androgen metabolism in the cells.[68] We confirmed that DHT converted from the androgen precursors is important in the cellular activity of the cancer cells. This shows that metabolism of the androgen in the prostate cancer cells plays an important role even in the exacerbation with androgen independent and androgen sensitive state after ADT. This conclusion is supported by the evidence that about 30% of recurrent prostate cancers show transient clinical responses to secondary or tertiary hormonal manipulation.[69] As a consequence, strategies to decrease adrenal cortical androgen production in patients with CRPC have been developed and are currently in widespread use. Ryan et al. revealed that higher androstenedione levels predict the likelihood of response to ketoconazole, which is used as an inhibitor of adrenal androgen synthesis, and improved survival compared with patients with lower levels. [70] These data suggest that the therapy with ketoconazole is less effective in patients with low levels of androgens at baseline. Ketoconazole therapy as secondary hormonal therapies may be less effective in the patients with low androgen levels in the prostate.

## **7. Clinical-translational advances of castration-resistant prostate cancer**

Recently, encouraging GnRH antagonists and early phase trial results of promising ADT agents that interfere with AR signaling for patients with hormone-naive prostate cancer or CRPC have been reported.

### **7.1 Gonadotrophin-releasing hormone (GnRH) antagonists: Degarelix**

Medical castration using GnRH agonists currently provides the mainstay of ADT for prostate cancer. Although effective, these agents only reduce testosterone levels after a delay of 14 to 21 days. [71] GnRH antagonists compete with natural GnRH for binding to GnRH receptors, thus decreasing or blocking GnRH action in the body. GnRH antagonists induce fast and profound LH and follicle-stimulating hormone (FSH) suppressions from the pituitary. In men, the reduction in LH subsequently leads to rapid suppression of testosterone release from the testes. Unlike the GnRH agonists, which cause an initial stimulation of the hypothalamic-pituitary-gonadal axis, leading to a surge in testosterone levels, and under certain circumstances, a flare-up of the tumor, GnRH antagonists do not cause a surge in testosterone or clinical flare. [72] Clinical flare is a phenomenon that occurs in patients with advanced disease, which can precipitate a range of clinical symptoms such as bone pain, ureteral obstruction, and spinal cord compression. As testosterone surge does not occur with GnRH antagonists, there is no need for patients to receive an antiandrogen as flare protection during prostate cancer treatment. GnRH agonists also induce an increase in

testosterone levels after each reinjection of the drug a phenomenon that does not occur with GnRH antagonists. GnRH antagonists have an immediate onset of action leading to a fast and profound suppression of testosterone and are therefore especially valuable in the treatment of patients with prostate cancer, where fast control of disease is needed. Abarelix, initial GnRH antagonist for prostate cancer, has been linked with immediate-onset systemic allergic reactions.[73] Degarelix is a new type of hormonal therapy for prostate cancer without stimulation of the release of histamine. [74] The reduction in LH subsequently leads to a rapid and sustained suppression of testosterone release from the testes and subsequently reduces the size and growth of the prostate cancer. This in turn results in a reduction in PSA levels in the patient's blood. Degarelix has an immediate onset of action, binding to GnRH receptors in the pituitary gland and blocking their interaction with GnRH. [75] The effectiveness of degarelix was recently evaluated in a Phase III, randomised, 12 month clinical trial (CS21) in 610 patients with prostate cancer, for whom ADT was indicated.[76] Patients were randomised to receive treatment with one of two doses of degarelix or the GnRH agonist, leuprolide. Both degarelix doses were at least as effective as leuprolide at suppressing testosterone to castration levels ( $\leq 0.5$  ng/mL) from Day 28 to study end (Day 364). Testosterone levels were suppressed significantly faster with degarelix than with leuprolide, with nearly all of the degarelix patients achieving castration levels by Day 3 of treatment compared with none in the leuprolide group. No patients receiving degarelix experienced a testosterone surge compared with 81% of those patients receiving leuprolide. Furthermore, degarelix resulted in a significantly faster reduction in PSA levels compared with leuprolide indicating faster control of the prostate cancer. Recent results also suggest that degarelix therapy may result in longer control of prostate cancer compared with leuprolide. [77] As with all hormonal therapies, degarelix is commonly associated with hormonal side effects such as hot flushes and weight gain. [76] Due to its mode of administration (subcutaneous injection), degarelix is also associated with injection-site reactions such as injection-site pain, erythema or swelling. Injection-site reactions are usually mild or moderate in intensity and occur predominantly after the first dose, decreasing in frequency thereafter.

### **7.2 Cytochrome P450, family 17, subfamily A, polypeptide 1 (CYP17A1) inhibitors**

The multifunctional  $17\alpha$ -hydroxylase/ $17,20$ -lyase encoded by Cytochrome P450, family 17, subfamily A, polypeptide 1 (CYP17A1) is a cytochrome P450 enzyme localized to the endoplasmic reticulum in the adrenals, testes, placenta and ovaries, and lies at the crossroads of sex steroid and glucocorticoid synthesis. It is encoded by a single gene found on chromosome 10 in humans. The enzyme possesses both  $17\alpha$ -hydroxylase activity and  $C17,20$ -lyase activity, and requires P450 reductase, cytochrome-b5 reductase (b5), to transfer electrons in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) for both catalytic activities. The degree of  $C17,20$ -lyase activity, modulated by cytochrome-b5, determines which metabolic pathway the substrate will follow in terms of sex steroid or glucocorticoid formation.[78, 79] Cytochrome-b5 is required for the  $C17,20$  lyase activity, and high b5/CYP17A1 ratios are associated with the nearly exclusive production of androgens in the testes as opposed to the low ratios observed in the adrenals where glucocorticoids are produced.[80, 81] As the critical catalytic step in all androgen biosynthesis, therapeutic CYP17A1 inhibition to treat prostate cancer and other androgen dependent diseases has been envisioned for over 50 years, with recent discoveries in

prostate cancer pathology driving much of the current refocus on CYP17A1 as a therapeutic target.[82, 83] Additionally, some evidence suggests that CYP17A1 mRNA and protein expression correlate with disease stage and relapse.[84, 85] Despite the strong rationale for targeting CYP17A1, this target has been largely unexploited with relatively few inhibitors progressing to clinical trials and only ketoconazole, an unspecific inhibitor of CYP17A1, in widespread clinical use.

### 7.3 Abiraterone acetate

One of CYP17A1 inhibitors, abiraterone acetate, the irreversible inhibitor of 17 $\alpha$ -hydroxylase/C17,20-lyase (CYP17A1), is well tolerated and has significant and durable anti-tumor activity both in patients with chemotherapy-naïve prostate cancer and in docetaxel-treated patients with CRPC. [86] In patients with CRPC, despite ADT, intra-tumoral androgen levels remain high, with continued AR signaling. The source of these androgens could be adrenal or de novo intra-tumoral synthesis. Researchers tried to inhibit CYP17A1, the key enzyme responsible for androgen synthesis, using abiraterone acetate, which is 10-fold or more potent of inhibition of CYP17A1 than ketoconazole, another inhibitor of CYP17A1. The phase II (open-label, single-arm) portion of the trial was an expansion of a promising phase I trial reported in 2008. [87] All 42 participants were castrated, and 38 had metastases, primarily in bone and/or lymph nodes. Their median age was 70. All received oral abiraterone acetate 1,000 mg daily in 28-day cycles. PSA declined at least 50% in 28 of the 42 patients (67%); eight patients (19%) had PSA decreases of at least 90%. Of the 24 patients who had measurable disease on CT scan, 9 (37.5%) had tumor regression that constituted a partial response. Further, 16 of these 24 patients (67%) showed no evidence of progression at 6 months. Baseline levels of DHEA, DHEA-S, androstenedione and estradiol were associated with the increased likelihood of a PSA decline of at least 50%. Adding dexamethasone 0.5 mg daily at the time of PSA progression reversed abiraterone acetate resistance in 33% of patients. This study and other phase II study revealed that abiraterone acetate has significant antitumor activity in post-docetaxel patients with CRPC. [88-90] Recent results of the phase III trial of abiraterone acetate in post-docetaxel patients has shown an overall survival benefit in advanced CRPC (COU-AA-301). [91] Abiraterone acetate is designed to treat these tumors by inhibiting the production of androgen in the testes, the adrenal glands and prostate cancer tumors themselves. The Phase III trial included 1195 patients from 13 countries whose metastatic, castration-resistant prostate cancer had previously been treated with one of two chemotherapeutic agents that included docetaxel. Among the 398 patients randomly assigned to receive the corticosteroid prednisone plus placebo, median overall survival was 10.9 months. Among the 797 who received abiraterone acetate plus prednisone, median survival was 14.8 months. Significant differences also emerged between the placebo and treatment groups for all of the trial's secondary endpoints, including time to PSA progression, radiographic progression-free survival and PSA response rate. The benefits of abiraterone were determined during a pre-specified interim analysis of the study, prompting the trial's Independent Data Monitoring Committee to recommend unblinding the trial, and allowing anyone on the placebo arm to be offered abiraterone acetate. Several ongoing trials are examining the agent in earlier settings (i.e., a phase III in metastatic CRPC pre-docetaxel, and smaller studies in combination with radiation therapy or as neoadjuvant pre-surgery for localized disease).

The US Food and Drug Administration (FDA) has approved abiraterone acetate (Zytiga, Cougar Biotechnology) in combination with prednisone for the treatment of metastatic CRPC in men who have received prior docetaxel chemotherapy. Herein, several potential strategies for abiraterone are presented to clarify the clinical utilization of this agent in the future. These evidences such as not only decline of PSA levels and the reduction of the tumor size but also the evidences such as the symptom improvement and improved survival duration are expected in future.

#### **7.4 Orteronel (TAK-700) [(1S)-1-(6,7-dimethoxy-2-naphthyl)-1-(1H-imidazol-4-yl)-2-methylpropan-1-ol]**

Orteronel is a non-steroidal imidazole CYP17A1 inhibitor ( $IC_{50} = 28nM$ ) currently in clinical trials in prostate cancer patients. The compound was found to be selective for CYP17A1 over  $11\beta$ -hydroxylase by an impressive 260-fold, and exhibited suppressive effects on testosterone biosynthesis in rats and reduction in the weight of prostate and seminal vesicles in the rat. [92, 93] Orteronel was also capable of reducing serum testosterone levels down to castration level after 8 hours following a single oral administration of 1mg/kg to monkeys. A practical asymmetric synthesis of Orteronel has been established in eight steps from 2,3-dihydroxy naphthalene. This phase I/II, open-label, dose-escalation study assesses the safety and tolerability of oral, twice-daily (BID) Orteronel in patients with metastatic CRPC. Secondary objectives include assessment of Orteronel efficacy, as shown by PSA response, and Orteronel pharmacokinetics. Orteronel  $\geq 300$  mg BID appears active and well tolerated in patients with metastatic CRPC. The recommended phase II dose is 400 mg BID. [ASCO 2010 Genitourinary Cancers Symposium] Orteronel is performed on two phase 3 clinical trials: one is a randomized, double-blind, multicenter, phase 3 study evaluating orteronel plus prednisone compared with placebo plus prednisone in the treatment of men with progressive, chemotherapy-naive, metastatic CRPC, the other is in men with metastatic CRPC that has progressed following taxane-based therapy.

#### **7.5 TOK-001 (VN/124-1) [3 $\beta$ -hydroxy-17-(1H-benzimidazole-1-yl)androsta-5,16-diene]**

The increased efficacy of TOK-001 in several prostate cancer models both in vitro and in vivo is believed to arise from its ability to down regulate the AR as well as competitively block androgen binding. [94, 95]. TOK-001 can act by several mechanisms (CYP17A1 inhibition, competitive inhibition and down-regulation of the AR). In prostate cancer cell lines, TOK-001 inhibited the growth of CRPCs, which had increased AR and were no longer sensitive to bicalutamide. Indeed, ARMOR1 (Androgen Receptor Modulation Optimized for Response1) phase 1/2 trials with TOK-001 was recently initiated in CRPC patients.

#### **7.6 3 $\beta$ -Hydroxysteroid dehydrogenase inhibitors**

Prostate cancer exhibits robust  $3\beta$ -HSD enzymatic activity, which is required for the AR-mediated response to  $\Delta$ -5 adrenal androgens and that  $3\beta$ -HSD may serve as a pharmacologic target for the treatment of CRPC. [96] Pharmacologic inhibition of  $3\beta$ -HSD might have therapeutic value regardless of the relative contribution of adrenal androgens as opposed to de novo steroidogenesis in the reconstitution of intratumoral testosterone and DHT. We showed that trilostane, a  $3\beta$ -HSD inhibitor, actually inhibits the in situ conversion of DHEA to androstenedione in prostate cancer cells, suggesting a new therapeutic target to more efficiently suppress intracellular androgen levels.[97] However, trilostane has a direct

androgenic ability in cancer cells with mutated or wild-type AR. Trilostane should be used with particular concern when treating prostate cancer, possibly suggesting the combination of trilostane and an AR antagonist such as bicalutamide or designing an inhibitor of 3 $\beta$ -HSD free of AR agonist activity.

### **7.7 Apoptone (HE3235) (17 $\alpha$ -ethynyl-5 $\alpha$ -androstan-3 $\alpha$ , 17 $\beta$ -diol)**

A novel androstane steroid, Apoptone (HE3235), an orally bioavailable synthetic analogue of 3 $\beta$ -androstenediol, has a significant inhibitory activity for 5'-androstenediol-stimulated LNCaP proliferation. [98, 99] Apoptone exhibits a wide range of effects, including alteration of androgen receptor signaling and reductions in levels of intratumoral androgens. This inhibitory activity is accompanied by an increase in the number of apoptotic cells. Animal studies have confirmed the cytoreductive activity of apoptone on LNCaP tumours. The results suggest that this compound may be of clinical use in CRPC. From the results of phase I/II clinical trial, apoptone is a novel, orally delivered agent with clinical activity in advanced CRPC patients. It is well tolerated and has an AUC linear to dose. Based on the safety and activity observed, the trial continues to accrue an expansion cohort at 100 mg/day of patients with chemotherapy-naive CRPC. [100]

### **7.8 Inhibitors targeting androgen receptor**

#### **7.8.1 A new androgen receptor antagonist, MDV3100 (Fig. 2)**

As for the conventional anti-androgen, affinity for AR and agonistic activity for AR are matters of concern. Because AR signaling appears to drive this cancer, blocking this signaling should stop its growth, the researchers theorized, and they went on to develop a drug that would do just that. Another novel ADT drug, AR antagonist MDV3100 (a diarylthiohydantoin) is a small-molecule, pure AR antagonist that inhibits AR nuclear translocation and DNA binding. [101, 102] MDV3100 is generally well tolerated, and shows encouraging anti-tumor activity in patients with CRPC. The drug was selected as a result of its activity in models of AR over-expression, in which conventional anti-androgens develop agonist properties. Currently available agents in the setting of AR over-expression actually act as agonists, rather than antagonists, so they actually make the disease worse, while MDV3100 has no known agonist activity. MDV3100 has demonstrated activity in models of bicalutamide-resistant prostate cancer. The results come from a phase 1/2 trial (NCT00510718) of 140 men, 65 of whom were chemotherapy-naive. Patients with progressive, metastatic CRPC were enrolled in dose-escalation cohorts of three to six patients and given an oral daily dose of MDV3100 from 30 mg to 600mg. The primary objective was to identify the safety and tolerability profile of MDV3100 and to establish the maximum tolerated dose. They noted antitumor effects at all doses, including decreases in serum prostate-specific antigen of 50% or more in 78 (56%) patients, responses in soft tissue in 13 (22%) of 59 patients, stabilized bone disease in 61 (56%) of 109 patients, and conversion from unfavorable to favorable circulating tumor cell counts in 25 (49%) of the 51 patients. PET imaging of 22 patients to assess androgen-receptor blockade showed decreased (18)F-fluoro-5 $\alpha$ -dihydrotestosterone binding at doses from 60 mg to 480 mg per day (range 20-100%). The median time to progression was 47 weeks (95% CI 34-not reached) for radiological progression. The maximum tolerated dose for sustained treatment (>28 days) was 240 mg. The most common grade 3-4 adverse event was dose-dependent fatigue (16 [11%] patients), which generally resolved after dose reduction. The encouraging antitumor

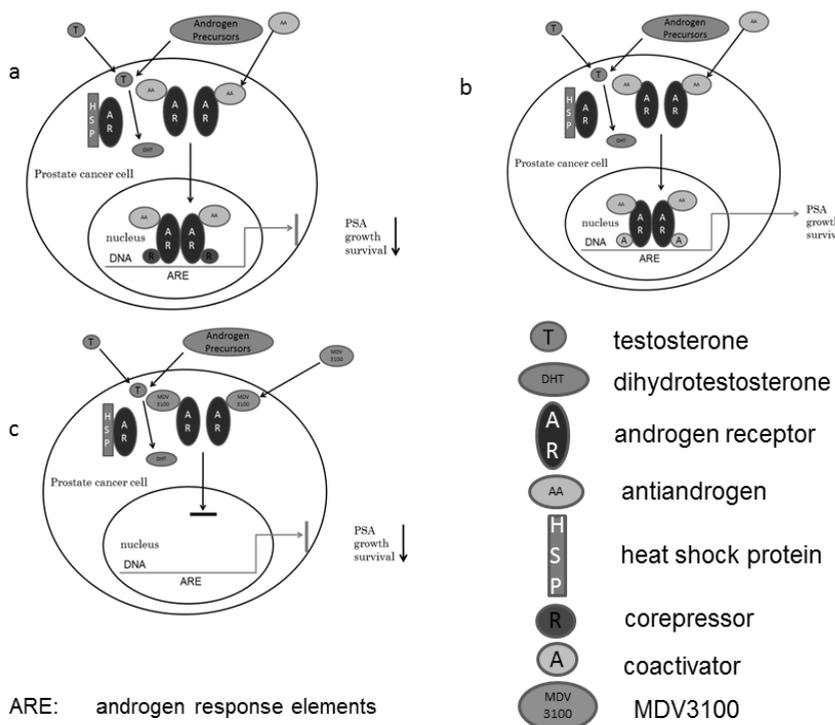


Fig. 2. The nuclear translocation and DNA binding on androgen receptors (AR) of androgens in the prostate cancer cells and action mechanisms of conventional AR antagonists and a new AR antagonist, MDV3100. (a) Conventional AR antagonists block AR with antagonistic action competitively in the prostate cancer cells. (b) Conventional AR antagonists showed agonistic activity in cells with increased AR levels; this antagonist-agonist conversion was associated with alterations in the recruitment of coactivators and corepressors to the promoters of AR target genes. Increased levels of AR confer resistance to anti-androgens by amplifying signal output from low levels of residual ligands, and by altering the normal response to antagonists. (c) MDV3100 is a pure AR antagonist that inhibits AR nuclear translocation and DNA binding. The affinity with AR of MDV3100 is stronger than that of conventional anti-androgens. MDV3100 has no known agonist activity for AR.

activity with MDV3100 in CRPC validates preclinical studies that have implicated AR signaling as a driver of this disease. MDV3100 is performed on two phase 3 clinical trials: one is a randomized, double-blind, multicenter, phase 3 study evaluating MDV3100 compared with placebo in the treatment of men with progressive, chemotherapy-naive, metastatic CRPC (PREVAIL), the other is in men with metastatic CRPC that has progressed following taxane-based therapy (AFFIRM).

ARN-509 is also an AR antagonist that inhibits nuclear translocation and DNA binding of the receptor, thereby modulating expression of genes that drive prostate cancer growth. Phase 1/2 clinical trial of ARN-509 in patients with CRPC was announced. (NCT01171898)

### **7.8.2 An inhibitor of the amino terminal domain (NTD) of the AR: EPI-001**

AR is activated by androgen or by other factors when androgen is removed, resulting in the transactivation of the amino terminal domain (NTD) of AR and AR binding to androgen response elements (AREs) in the promoters of androgen-regulated genes. All current therapies that target the AR are dependent on the presence of its C-terminal ligand-binding domain (LBD). A library of extracts from marine sponges for an inhibitor of AR NTD transactivation were screened and the small molecule EPI-001 was identified, which inhibited transcriptional regulation of androgen-regulated genes, but not those regulated by the related progesterone and glucocorticoid steroid receptors. [103] Importantly, EPI-001 was effective at inhibiting both ligand-dependent and ligand-independent transactivation of AR, as well as inhibiting constitutively active AR splice variants that are devoid of LBD. EPI-001 has a new mode of action, as it targets the transactivation of AR regardless of the presence of androgen, and is a promising therapy to delay the progression of CRPC.

## **8. The prospects of the androgen deprivation therapy on the ground of androgen milieu in the prostate**

Conventional ADTs that we have used cannot decrease DHT levels in the prostate sufficiently to restrain the growth of the cancer in patient with aggressive prostate cancer, as we mentioned above. The androgen levels in blood were low; however, it became clear that the AR signals play important roles for progress of the cancers from the studies of the prostate cancer that became exacerbation after ADT. The androgen milieu in men with high Gleason score prostate cancer is probably less affected by conventional ADT than that in men with low score cancer, which was suggested to be associated with adrenal androgen levels. In patients treated with ADT the pituitary-adrenal axis mediated by adrenocorticotrophic hormone has a central role in the regulation of androgen synthesis. Adrenocorticotrophic hormone mediated androgen synthesis is a potential target for advanced ADT. Importantly, the suboptimal suppression of androgen activity in the prostate cancer may account for the heterogeneity in treatment effect observed across individual patients, and, moreover, may contribute to the outgrowth of resistant prostate cancer clones adapted to survive in a low androgen environment, suggesting that more effective methods for suppressing the androgen axis in the tumoral microenvironment are required. From the results of the high reactivity of a CYP17A1 inhibitor and a new AR antagonist for patients with CRPC, the androgen syntheses and the maintenances of the AR signal system in the prostate cancer play important roles in etiological mechanisms of CRPC. As for the ADT for the patients with aggressive prostate cancer, it is important to restrain productions of testosterone and DHT and to restrain AR activity together with castration from early period of treatment. The results with new ADT agents, such as MDV3100 and abiraterone acetate seem to validate the hypothesis that androgens and the AR are vital to progression of metastatic CRPC.

## **9. Conclusions**

The patients with prostate cancer of high Gleason score have low DHT levels in the prostatic tissue. Biologically aggressive prostate cancer can occur under a low DHT level environment where the prostate cancer of a low malignancy with high DHT dependency cannot easily occur. Conventional ADTs that we have used cannot decrease DHT levels in

the prostate sufficiently to restrain the growth of the cancer in patients with aggressive prostate cancer. Persistent levels of DHT in the prostate after castration are derived from adrenal androgens in the prostate. The androgen milieu in men with high Gleason score prostate cancer is probably less affected by conventional ADT than that in men with low score cancer, which was suggested to be associated with adrenal androgen levels. In patients treated with ADT the pituitary-adrenal axis mediated by adrenocorticotrophic hormone has a central role in the regulation of androgen synthesis. These locally produced androgens can play important roles in the pathogenesis and development of prostate cancer. AR signaling pathway is an important mechanism in proliferation of aggressive prostate cancer during ADT. AR transcriptional activity is reactivated in CRPC and identifies the intracellular conversion of adrenal androgens to DHT as a mechanism mediating this reactivation. New ADTs using CYP17A1 inhibitors such as abiraterone acetate and AR antagonists such as MDV3100 for patients with CRPC, which will break through the limitation of the efficacy of conventional ADTs that have been used for nearly 70 years, have been developing. Therefore, it is important to examine the status of in situ androgen metabolism and/or the AR signal system in detail in order to improve the clinical response to ADT in patients diagnosed with biologically aggressive prostate cancer from now on.

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## 11. References

- [1] Heinlein CA, Chang C., Androgen receptor in prostate cancer., *Endocr Rev.* 2004;25(2):276-308.
- [2] Huggins C, Hodges CV. Studies on prostate cancer. Effect of castration, estrogen and androgen injection on serum phosphatases in metastatic carcinoma of the prostate. *Cancer Res* 1941; 1:293-7.
- [3] McLeod DG, Crawford ED, DeAntoni EP. Combined androgen blockade: the gold standard for metastatic prostate cancer. *Eur Urol.* 1997;32 Suppl 3:70-7.
- [4] Pienta KJ, Bradley D. Mechanisms underlying the development of androgen-independent prostate cancer. *Clin Cancer Res.* 2006; 12: 1665-71.
- [5] Di Lorenzo G, Buonerba C, Autorino R, De Placido S, Sternberg CN. Castration-resistant prostate cancer: current and emerging treatment strategies. *Drugs.* 2010;70(8):983-1000.
- [6] Winters SJ, Clark BJ. Testosterone Synthesis, Transport, and Metabolism. In Bagatell CJ, Bremner WJ (eds), *Androgens in Health and Disease.* pp3-22. Humana Press, 2003.
- [7] Rommerts FFG. Testosterone: overview of biosynthesis, transport, metabolism and non-genomic actions. In Nieschlag E, Behre HM, Niechlag S (eds) *Testosterone.* 3rd edn, pp1-38. Cambridge, 2004.
- [8] Haider SG., Cell biology of Leydig cells in the testis., *Int Rev Cytol.* 2004;233:181-241.
- [9] Midzak AS, Chen H, Papadopoulos V, Zirkin BR. Leydig cell aging and the mechanisms of reduced testosterone synthesis. *Mol Cell Endocrinol.* 2009;299(1):23-31.

- [10] Endogenous Hormones and Prostate Cancer Collaborative Group, Roddam AW, Allen NE, Appleby P, Key TJ., Endogenous sex hormones and prostate cancer: a collaborative analysis of 18 prospective studies., *J Natl Cancer Inst.* 2008;100(3):170-83.
- [11] Morote J, Ramirez C, Gomez E, Planas J, Raventos CX, de Torres IM, Catalan R., The relationship between total and free serum testosterone and the risk of prostate cancer and tumour aggressiveness., *BJU Int.* 2009;104(4):486-9.
- [12] Luu-The V, Belanger A, Labrie F., Androgen biosynthetic pathways in the human prostate., *Best Pract Res Clin Endocrinol Metab.* 2008;22(2):207-21.
- [13] Penning TM, Byrns MC., Steroid hormone transforming aldo-keto reductases and cancer., *Ann N Y Acad Sci.* 2009;1155:33-42.
- [14] Ghayee HK, Auchus RJ., Clinical implications of androgen synthesis via 5 $\alpha$ -reduced precursors., *Endocr Dev.* 2008;13:55-66.
- [15] Zhu YS, Imperato-McGinley JL., 5 $\alpha$ -reductase isozymes and androgen actions in the prostate., *Ann N Y Acad Sci.* 2009;1155:43-56.
- [16] Uemura M, Tamura K, Chung S, Honma S, Okuyama A, Nakamura Y, Nakagawa H. Novel 5  $\alpha$ -steroid reductase (SRD5A3, type-3) is overexpressed in hormone-refractory prostate cancer. *Cancer Sci.* 2008;99(1):81-6.
- [17] Godoy A, Kawinski E, Li Y, Oka D, Alexiev B, Azzouni F, Titus MA, Mohler JL. 5 $\alpha$ -reductase type 3 expression in human benign and malignant tissues: A comparative analysis during prostate cancer progression. *Prostate.* 2010 Dec 28. [Epub ahead of print]
- [18] Auchus RJ., The backdoor pathway to dihydrotestosterone., *Trends Endocrinol Metab.* 2004;15(9):432-8.
- [19] Ghayee HK, Auchus RJ., Basic concepts and recent developments in human steroid hormone biosynthesis., *Rev Endocr Metab Disord.* 2007;8(4):289-300.
- [20] Sharifi N, McPhaul MJ, Auchus RJ. "Getting from here to there"--mechanisms and limitations to the activation of the androgen receptor in castration-resistant prostate cancer. *J Investig Med.* 2010;58(8):938-44.
- [21] Morgentaler A., Testosterone and prostate cancer: an historical perspective on a modern myth., *Eur Urol.* 2006;50(5):935-9.
- [22] Isbarn H, Pinthus JH, Marks LS, Montorsi F, Morales A, Morgentaler A, Schulman C., Testosterone and prostate cancer: revisiting old paradigms., *Eur Urol.* 2009;56(1):48-56.
- [23] Morgentaler A, Traish AM., Shifting the paradigm of testosterone and prostate cancer: the saturation model and the limits of androgen-dependent growth., *Eur Urol.* 2009;55(2):310-20.
- [24] Weiss JM, Huang WY, Rinaldi S, Fears TR, Chatterjee N, Hsing AW, Crawford ED, Andriole GL, Kaaks R, Hayes RB. Endogenous sex hormones and the risk of prostate cancer: a prospective study. *Int J Cancer.* 2008;122(10):2345-50.
- [25] Bartsch W, Klein H, Schiemann U, Bauer HW, Voigt KD. Enzymes of androgen formation and degradation in the human prostate. *Ann N Y Acad Sci.* 1990;595:53-66.
- [26] Nishiyama T, Hashimoto Y, Takahashi K. The influence of androgen deprivation therapy on dihydrotestosterone levels in the prostatic tissue of patients with prostate cancer. *Clin Cancer Res.* 2004;10(21):7121-6.

- [27] Nishiyama T, Ikarashi T, Hashimoto Y, Suzuki K, Takahashi K. Association between the dihydrotestosterone level in the prostate and prostate cancer aggressiveness using the Gleason score. *J Urol*. 2006;176(4 Pt 1):1387-91.
- [28] Prehn RT. On the prevention and therapy of prostate cancer by androgen administration. *Cancer Res*. 1999;59(17):4161-4.
- [29] Freedland SJ, Isaacs WB, Platz EA, Terris MK, Aronson WJ, Amling CL, Presti JC Jr, Kane CJ. Prostate size and risk of high-grade, advanced prostate cancer and biochemical progression after radical prostatectomy: a search database study. *J Clin Oncol*. 2005;23(30):7546-54.
- [30] Kwon T, Jeong IG, You D, Park MC, Hong JH, Ahn H, Kim CS. Effect of prostate size on pathological outcome and biochemical recurrence after radical prostatectomy for prostate cancer: is it correlated with serum testosterone level? *BJU Int*. 2010;106(5):633-8.
- [31] Newton MR, Phillips S, Chang SS, Clark PE, Cookson MS, Davis R, Fowke JH, Herrell SD, Baumgartner R, Chan R, Mishra V, Blume JD, Smith JA Jr, Barocas DA. Smaller prostate size predicts high grade prostate cancer at final pathology. *J Urol*. 2010;184(3):930-7.
- [32] Thompson IM, Goodman PJ, Tangen CM, Lucia MS, Miller GJ, Ford LG, Lieber MM, Cespedes RD, Atkins JN, Lippman SM, Carlin SM, Ryan A, Szczepanek CM, Crowley JJ, Coltman CA Jr. The influence of finasteride on the development of prostate cancer. *N Engl J Med*. 2003;349(3):215-24.
- [33] Rubin MA, Allory Y, Molinie V, Leroy X, Faucon H, Vacherot F, Huang W, Kuten A, Salomon L, Rebillard X, Cussenot O, Abbou C, de la Taille A. Effects of long-term finasteride treatment on prostate cancer morphology and clinical outcome. *Urology*. 2005;66(5):930-4.
- [34] Lucia MS, Epstein JI, Goodman PJ, Darke AK, Reuter VE, Civantos F, Tangen CM, Parnes HL, Lippman SM, La Rosa FG, Kattan MW, Crawford ED, Ford LG, Coltman CA Jr, Thompson IM. Finasteride and high-grade prostate cancer in the Prostate Cancer Prevention Trial. *J Natl Cancer Inst*. 2007;99(18):1375-83.
- [35] Andriole GL, Bostwick DG, Brawley OW, Gomella LG, Marberger M, Montorsi F, Pettaway CA, Tammela TL, Teloken C, Tindall DJ, Somerville MC, Wilson TH, Fowler IL, Rittmaster RS; REDUCE Study Group. Effect of dutasteride on the risk of prostate cancer. *N Engl J Med*. 2010 Apr 1;362(13):1192-202.
- [36] Li R, Wheeler T, Dai H, Frolov A, Thompson T, Ayala G. High level of androgen receptor is associated with aggressive clinicopathologic features and decreased biochemical recurrence-free survival in prostate: cancer patients treated with radical prostatectomy. *Am J Surg Pathol*. 2004;28(7):928-34.
- [37] Fung KM, Samara EN, Wong C, Metwalli A, Krilin R, Bane B, Liu CZ, Yang JT, Pitha JV, Culkun DJ, Kropp BP, Penning TM, Lin HK. Increased expression of type 2  $3\alpha$ -hydroxysteroid dehydrogenase/type 5  $17\beta$ -hydroxysteroid dehydrogenase (AKR1C3) and its relationship with androgen receptor in prostate carcinoma. *Endocr Relat Cancer*. 2006;13(1):169-80.
- [38] Wako K, Kawasaki T, Yamana K, Suzuki K, Jiang S, Umezumi H, Nishiyama T, Takahashi K, Hamakubo T, Kodama T, Naito M. Expression of androgen receptor through androgen-converting enzymes is associated with biological aggressiveness in prostate cancer. *J Clin Pathol*. 2008;61(4):448-54.

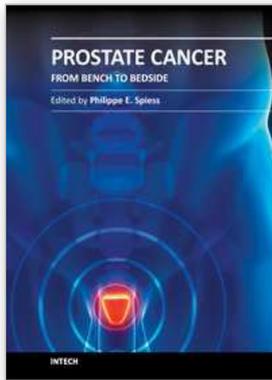
- [39] Maatman TJ, Gupta MK, Montie JE. Effectiveness of castration versus intravenous estrogen therapy in producing rapid endocrine control of metastatic cancer of the prostate. *J Urol.* 1985;133:620-1.
- [40] Oefelein MG, Feng A, Scolieri MJ, Ricchiutti D and Resnick MI Reassessment of the definition of castrate levels of testosterone: implications for clinical decision making. *Urology* 2000; 56: 1021-1024.
- [41] Tammela T. Endocrine treatment of prostate cancer. *J Steroid Biochem Mol Biol.* 2004;92:287-95.
- [42] Tombal B. Appropriate Castration with Luteinising Hormone Releasing Hormone (LHRH) Agonists: What is the Optimal Level of Testosterone? *Eur Urol Suppl* 2005; 4:14-9.
- [43] Crawford ED, Rove KO. Incomplete testosterone suppression in prostate cancer. *N Engl J Med.* 2010;363(20):1976.
- [44] Morote J, Orsola A, Planas J, Trilla E, Raventos CX, Cecchini L, Catalan R. Redefining clinically significant castration levels in patients with prostate cancer receiving continuous androgen deprivation therapy. *J Urol.* 2007 ;178(4 Pt 1) :1290-5.
- [45] Perachino M, Cavalli V, Bravi F. Testosterone levels in patients with metastatic prostate cancer treated with luteinizing hormone-releasing hormone therapy: prognostic significance? *BJU Int.* 2010 Mar;105(5):648-51.
- [46] Labrie F, Belanger A, Luu-The V, Labrie C, Simard J, Cusan L, Gomez J, Candas B. Gonadotropin-releasing hormone agonists in the treatment of prostate cancer. *Endocr Rev.* 2005; 26: 361-79.
- [47] Labrie F, Belanger A, Dupont A, Luu-The V, Simard J, Labrie C. Science behind total androgen blockade: from gene to combination therapy. *Clin Invest Med.* 1993; 16: 475-92.
- [48] Hammond GL. Endogenous steroid levels in the human prostate from birth to old age: a comparison of normal and diseased tissues. *J Endocrinol.* 1978; 78: 7-19.
- [49] Belanger B, Belanger A, Labrie F, Dupont A, Cusan L, Monfette G. Comparison of residual C-19 steroids in plasma and prostatic tissue of human, rat and guinea pig after castration: unique importance of extratesticular androgens in men. *J Steroid Biochem.* 1989; 32: 695-8.
- [50] Geller J, Albert JD. Effects of castration compared with total androgen blockade on tissue dihydrotestosterone (DHT) concentration in benign prostatic hyperplasia (BPH). *Urol Res.* 1987; 15: 151-3.
- [51] Nishiyama T, Ikarashi T, Hashimoto Y, Wako K, Takahashi K. The change in the dihydrotestosterone level in the prostate before and after androgen deprivation therapy in connection with prostate cancer aggressiveness using the Gleason score. *J Urol.* 2007;178(4 Pt 1):1282-8.
- [52] Titus MA, Gregory CW, Ford OH 3rd, Schell MJ, Maygarden SJ, Mohler JL. Steroid 5 $\alpha$ -reductase isozymes I and II in recurrent prostate cancer. *Clin Cancer Res.* 2005;11(12):4365-71.
- [53] Thomas LN, Lazier CB, Gupta R, Norman RW, Troyer DA, O'Brien SP, Rittmaster RS. Differential alterations in 5 $\alpha$ -reductase type 1 and type 2 levels during development and progression of prostate cancer. *Prostate.* 2005;63:231-9.

- [54] Arai S, Miyashiro Y, Shibata Y, Tomaru Y, Kobayashi M, Honma S, Suzuki K. Effect of castration monotherapy on the levels of adrenal androgens in cancerous prostatic tissues. *Steroids*. 2011 Feb;76(3):301-8.
- [55] Grossmann ME, Huang H, Tindall DJ. Androgen receptor signaling in androgen-refractory prostate cancer. *J Natl Cancer Inst*. 2001 ;93 :1687-97.
- [56] 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(5):2815-25.
- [57] Mostaghel EA, Page ST, Lin DW, Fazli L, Coleman IM, True LD, Knudsen B, Hess DL, Nelson CC, Matsumoto AM, Bremner WJ, Gleave ME, Nelson PS. Intraprostatic androgens and androgen-regulated gene expression persist after testosterone suppression: therapeutic implications for castration-resistant prostate cancer. *Cancer Res*. 2007;67:5033-41.
- [58] Takizawa I, Nishiyama T, Hara N, Isahaya E, Hoshii T, Takahashi K. Serum prostate-specific antigen levels reflect the androgen milieu in patients with localized prostate cancer receiving androgen deprivation therapy: Tumor malignant potential and androgen milieu., *Prostate*. 2010;70(13):1395-401.
- [59] Takizawa I, Hara N, Nishiyama T, Isahaya E, Hoshii T, Takahashi K. Adrenocorticotrophic hormone is involved in regulation of androgen synthesis in men receiving androgen deprivation therapy for localized prostate cancer. *J Urol*. 2010;184(5):1971-6.
- [60] Attar RM, Takimoto CH, Gottardis MM. Castration-resistant prostate cancer: locking up the molecular escape routes. *Clin Cancer Res*. 2009;15(10):3251-5.
- [61] Yuan X, Balk SP. Mechanisms mediating androgen receptor reactivation after castration. *Urol Oncol*. 2009;27(1):36-41.
- [62] 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(1):33-9.
- [63] Mohler JL, Gregory CW, Ford OH 3rd, Kim D, Weaver CM, Petrusz P, Wilson EM, French FS. The androgen axis in recurrent prostate cancer. *Clin Cancer Res*. 2004;10(2):440-8.
- [64] Titus MA, Schell MJ, Lih FB, Tomer KB, Mohler JL. Testosterone and dihydrotestosterone tissue levels in recurrent prostate cancer. *Clin Cancer Res*. 2005;11(13):4653-7.
- [65] Montgomery RB, Mostaghel EA, Vessella R, Hess DL, Kalthorn 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(11):4447-54.
- [66] Locke JA, Guns ES, Lubik AA, Adomat HH, Hendy SC, Wood CA, Ettinger SL, Gleave ME, Nelson CC. Androgen levels increase by intratumoral de novo steroidogenesis during progression of castration-resistant prostate cancer. *Cancer Res*. 2008;68(15):6407-15.
- [67] Dillard PR, Lin MF, Khan SA. Androgen-independent prostate cancer cells acquire the complete steroidogenic potential of synthesizing testosterone from cholesterol. *Mol Cell Endocrinol*. 2008;295(1-2):115-20.

- [68] Suzuki K, Nishiyama T, Hara N, Yamana K, Takahashi K, Labrie F. Importance of the intracrine metabolism of adrenal androgens in androgen-dependent prostate cancer. *Prostate Cancer Prostatic Dis.* 2007;10(3):301-6.
- [69] Lam JS, Leppert JT, Vemulapalli SN, Shvarts O, Belldegrin AS. Secondary hormonal therapy for advanced prostate cancer. *J Urol.* 2006;175(1):27-34.
- [70] Ryan CJ, Halabi S, Ou SS, Vogelzang NJ, Kantoff P, Small EJ. Adrenal androgen levels as predictors of outcome in prostate cancer patients treated with ketoconazole plus antiandrogen withdrawal: results from a cancer and leukemia group B study. *Clin Cancer Res.* 2007;13(7):2030-7.
- [71] Van Poppel H. Evaluation of degarelix in the management of prostate cancer. *Cancer Manag Res.* 2010 Jan 25;2:39-52.
- [72] van Poppel H, Nilsson S. Testosterone surge: rationale for gonadotropin-releasing hormone blockers? *Urology.* 2008 Jun;71(6):1001-6.
- [73] Debruyne F, Bhat G, Garnick MB. Abarelix for injectable suspension: first-in-class gonadotropin-releasing hormone antagonist for prostate cancer. *Future Oncol.* 2006 Dec;2(6):677-96.
- [74] Koechling W, Hjortkjaer R, Tanko LB. Degarelix, a novel GnRH antagonist, causes minimal histamine release compared with cetorelix, abarelix and ganirelix in an ex vivo model of human skin samples. *Br J Clin Pharmacol.* 2010 Oct;70(4):580-7.
- [75] Princivalle M, Broqua P, White R, Meyer J, Mayer G, Elliott L, Bjarnason K, Haigh R, Yea C. Rapid suppression of plasma testosterone levels and tumor growth in the dunning rat model treated with degarelix, a new gonadotropin-releasing hormone antagonist. *J Pharmacol Exp Ther.* 2007 Mar;320(3):1113-8.
- [76] Klotz L, Boccon-Gibod L, Shore ND, Andreou C, Persson BE, Cantor P, Jensen JK, Olesen TK, Schroder FH. The efficacy and safety of degarelix: a 12-month, comparative, randomized, open-label, parallel-group phase III study in patients with prostate cancer. *BJU Int.* 2008 Dec;102(11):1531-8.
- [77] Tombal B, Miller K, Boccon-Gibod L, Schroder F, Shore N, Crawford ED, Moul J, Jensen JK, Kold Olesen T, Persson BE. Additional Analysis of the Secondary End Point of Biochemical Recurrence Rate in a Phase 3 Trial (CS21) Comparing Degarelix 80mg Versus Leuprolide in Prostate Cancer Patients Segmented by Baseline Characteristics. *Eur Urol.* 2010 May;57(5):836-42.
- [78] Lee-Robichaud P, Shyadehi AZ, Wright JN, Akhtar ME, Akhtar M. Mechanistic kinship between hydroxylation and desaturation reactions: acyl-carbon bond cleavage promoted by pig and human CYP17 (P-450(17) $\alpha$ ; 17  $\alpha$ -hydroxylase-17,20-lyase). *Biochemistry.* 1995;34(43):14104-13.
- [79] Akhtar MK, Kelly SL, Kaderbhai MA. Cytochrome b(5) modulation of 17 $\alpha$ -hydroxylase and 17-20 lyase (CYP17) activities in steroidogenesis. *J Endocrinol.* 2005;187(2):267-74.
- [80] Katagiri M, Kagawa N, Waterman MR. The role of cytochrome b5 in the biosynthesis of androgens by human P450c17. *Arch Biochem Biophys.* 1995;317(2):343-7.
- [81] Dharia S, Slane A, Jian M, Conner M, Conley AJ, Parker CR Jr. Colocalization of P450c17 and cytochrome b5 in androgen-synthesizing tissues of the human. *Biol Reprod.* 2004;71(1):83-8.
- [82] Gaunt R, Steinetz BG, Chart JJ. Pharmacologic alteration of steroid hormone functions. *Clin Pharmacol Ther.* 1968;9(5):657-81.

- [83] Arth GE, Patchett AA, Jefopoulos T, Bugianesi RL, Peterson LH, Ham EA, Kuehl FA Jr, Brink NG. Steroidal androgen biosynthesis inhibitors. *J Med Chem.* 1971;14(8):675-9.
- [84] Montgomery RB, Mostaghel EA, Vessella R, Hess DL, Kalthorn 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(11):4447-54.
- [85] Stigliano A, Gandini O, Cerquetti L, Gazzaniga P, Misiti S, Monti S, Gradilone A, Falasca P, Poggi M, Brunetti E, Agliano AM, Toscano V. Increased metastatic lymph node 64 and CYP17A1 expression are associated with high stage prostate cancer. *J Endocrinol.* 2007;194(1):55-61.
- [86] Attard G, Reid AH, Yap TA, Raynaud F, Dowsett M, Settatree S, Barrett M, Parker C, Martins V, Folkerd E, Clark J, Cooper CS, Kaye SB, Dearnaley D, Lee G, de Bono JS. Phase I clinical trial of a selective inhibitor of CYP17, abiraterone acetate, confirms that castration-resistant prostate cancer commonly remains hormone driven. *J Clin Oncol.* 2008;26(28):4563-71.
- [87] Attard G, Reid AH, A'Hern R, Parker C, Oommen NB, Folkerd E, Messiou C, Molife LR, Maier G, Thompson E, Olmos D, Sinha R, Lee G, Dowsett M, Kaye SB, Dearnaley D, Kheoh T, Molina A, de Bono JS. Selective inhibition of CYP17 with abiraterone acetate is highly active in the treatment of castration-resistant prostate cancer. *J Clin Oncol.* 2009;27(23):3742-8.
- [88] Danila DC, Morris MJ, de Bono JS, Ryan CJ, Denmeade SR, Smith MR, Taplin ME, Bublely GJ, Kheoh T, Haqq C, Molina A, Anand A, Kosciuszka M, Larson SM, Schwartz LH, Fleisher M, Scher HI. Phase II multicenter study of abiraterone acetate plus prednisone therapy in patients with docetaxel-treated castration-resistant prostate cancer. *J Clin Oncol.* 2010;28(9):1496-501.
- [89] Reid AH, Attard G, Danila DC, Oommen NB, Olmos D, Fong PC, Molife LR, Hunt J, Messiou C, Parker C, Dearnaley D, Swennenhuis JF, Terstappen LW, Lee G, Kheoh T, Molina A, Ryan CJ, Small E, Scher HI, de Bono JS. Significant and sustained antitumor activity in post-docetaxel, castration-resistant prostate cancer with the CYP17 inhibitor abiraterone acetate. *J Clin Oncol.* 2010;28(9):1489-95.
- [90] Ryan CJ, Smith MR, Fong L, Rosenberg JE, Kantoff P, Raynaud F, Martins V, Lee G, Kheoh T, Kim J, Molina A, Small EJ. Phase I clinical trial of the CYP17 inhibitor abiraterone acetate demonstrating clinical activity in patients with castration-resistant prostate cancer who received prior ketoconazole therapy. *J Clin Oncol.* 2010;28(9):1481-8.
- [91] 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(21):1995-2005.
- [92] Matsunaga N, Kaku T, Itoh F, Tanaka T, Hara T, Miki H, Iwasaki M, Aono T, Yamaoka M, Kusaka M, Tasaka A. C17,20-lyase inhibitors I. Structure-based de novo design and SAR study of C17,20-lyase inhibitors. *Bioorg Med Chem.* 2004;12(9):2251-73.
- [93] Matsunaga N, Kaku T, Ojida A, Tanaka T, Hara T, Yamaoka M, Kusaka M, Tasaka A. C(17,20)-lyase inhibitors. Part 2: design, synthesis and structure-activity

- relationships of (2-naphthylmethyl)-1H-imidazoles as novel C(17,20)-lyase inhibitors. *Bioorg Med Chem*. 2004;12(16):4313-36.
- [94] Handratta VD, Vasaitis TS, Njar VC, Gediya LK, Kataria R, Chopra P, Newman D Jr, Farquhar R, Guo Z, Qiu Y, Brodie AM. Novel C-17-heteroaryl steroidal CYP17 inhibitors/antiandrogens: synthesis, in vitro biological activity, pharmacokinetics, and antitumor activity in the LAPC4 human prostate cancer xenograft model. *J Med Chem*. 2005;48(8):2972-84.
- [95] Vasaitis T, Belosay A, Schayowitz A, Khandelwal A, Chopra P, Gediya LK, Guo Z, Fang HB, Njar VC, Brodie AM. Androgen receptor inactivation contributes to antitumor efficacy of 17 $\alpha$ -hydroxylase/17,20-lyase inhibitor 3 $\beta$ -hydroxy-17-(1H-benzimidazole-1-yl)androsta-5,16-diene in prostate cancer. *Mol Cancer Ther*. 2008;7(8):2348-57.
- [96] Evaul K, Li R, Papari-Zareei M, Auchus RJ, Sharifi N. 3 $\beta$ -hydroxysteroid dehydrogenase is a possible pharmacological target in the treatment of castration-resistant prostate cancer. *Endocrinology*. 2010;151(8):3514-20.
- [97] Takizawa I, Nishiyama T, Hara N, Hoshii T, Ishizaki F, Miyashiro Y, Takahashi K. Trilostane, an inhibitor of 3 $\beta$ -hydroxysteroid dehydrogenase, has an agonistic activity on androgen receptor in human prostate cancer cells. *Cancer Lett*. 2010;297(2):226-30.
- [98] Trauger R, Corey E, Bell D, White S, Garsd A, Stickney D, Reading C, Frincke J. Inhibition of androstenediol-dependent LNCaP tumour growth by 17 $\alpha$ -ethynyl-5 $\alpha$ -androstane-3 $\alpha$ , 17 $\beta$ -diol (HE3235). *Br J Cancer*. 2009;100(7):1068-72.
- [99] Koreckij TD, Trauger RJ, Montgomery RB, Pitts TE, Coleman I, Nguyen H, Reading CL, Nelson PS, Vessella RL, Corey E. HE3235 inhibits growth of castration-resistant prostate cancer. *Neoplasia*. 2009;11(11):1216-25.
- [100] Ahlem C, Kennedy M, Page T, Bell D, Delorme E, Villegas S, Reading C, White S, Stickney D, Frincke J. 17 $\alpha$ -Alkynyl 3 $\alpha$ , 17 $\beta$ -androstenediol non-clinical and clinical pharmacology, pharmacokinetics and metabolism. *Invest New Drugs*. 2010 Sep 3. [Epub ahead of print]
- [101] Tran C, Ouk S, Clegg NJ, Chen Y, Watson PA, Arora V, Wongvipat J, Smith-Jones PM, Yoo D, Kwon A, Wasielewska T, Welsbie D, Chen CD, Higano CS, Beer TM, Hung DT, Scher HI, Jung ME, Sawyers CL. Development of a second-generation antiandrogen for treatment of advanced prostate cancer. *Science*. 2009;324(5928):787-90.
- [102] Scher HI, Beer TM, Higano CS, Anand A, Taplin ME, Efstathiou E, Rathkopf D, Shelkey J, Yu EY, Alumkal J, Hung D, Hirmand M, Seely L, Morris MJ, Danila DC, Humm J, Larson S, Fleisher M, Sawyers CL; Prostate Cancer Foundation/Department of Defense Prostate Cancer Clinical Trials Consortium. Antitumour activity of MDV3100 in castration-resistant prostate cancer: a phase 1-2 study. *Lancet*. 2010;375(9724):1437-46.
- [103] Andersen, R. J. et al. Regression of castrate-recurrent prostate cancer by a small-molecule inhibitor of the amino-terminus domain of the androgen receptor. *Cancer Cell* 2010;17: 535-546.



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The present textbook highlights many of the exciting discoveries made in the diagnosis and treatment of prostate cancer over the past decade. International thought leaders have contributed to this effort providing a comprehensive and state-of-the art review of the signaling pathways and genetic alterations essential in prostate cancer. This work provides an essential resource for healthcare professionals and scientists dedicated to this field. This textbook is dedicated to the efforts and advances made by our scientific community, realizing we have much to learn in striving to some day in the not too distant future cure this disease particularly among those with an aggressive tumor biology.

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