Chapter I

Androgen Deprivation Therapy for Advanced Prostate Cancer on the Ground of Androgen Milieu in the Prostate

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Abstract

Androgens and the androgen receptor (AR) have been implicated in aggressiveness of prostate cancer. Huggins and Hodges in 1941 demonstrated that bilateral orchiectomy or estrogen treatment is an effective treatment for prostate cancer. Based upon their findings, androgen deprivation therapy (ADT) has remained the main therapeutic option for patients with advanced prostate cancer for about 70 years. 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. The serum testosterone level in men being treated with ADT has never been categorically established. 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. 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. 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

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high Gleason score prostate cancer is probably less affected by conventional ADT than that in men with low Gleason score cancer, which was suggested to be associated with adrenal androgen levels. In patients treated with ADT the pituitary-adrenal axis mediated by adrenocorticotropic hormone has a central role in the regulation of androgen synthesis. Importantly, the suboptimal suppression of androgen activity in prostate cancer may account for the heterogeneity in treatment effect observed across individual patients, 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 tumor microenvironment are required. From the results of the high reactivity of CYP17A1 inhibitors and a new AR antagonist for patients with castration-resistant prostate cancer (CRPC), the androgen syntheses and the maintenances of the AR signal system in the prostate cancer play important roles in etiological mechanisms of CRPC. AR transcriptional activity is reactivated in CRPC and identifies the intracellular conversion of adrenal androgens to DHT as a mechanism mediating this reactivation. 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. 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.

Abbreviations

ADT	androgen deprivation therapy
AKR1C3	Aldo-keto reductase family 1, member C3
AR	androgen receptor
b5	cytochrome-b5 reductase
CRPC	castration-resistant prostate cancer
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CYP17A1	Cytochrome P450, family 17, subfamily A, polypeptide 1
DHEA	dehydroepiandrosterone
DHEA-S	DHEA sulfate
DHT	dihydrotestosterone
FSH	follicle-stimulating hormone
GnRH	gonadotropin-releasing hormone
HSD	hydroxysteroid dehydrogenase
LH	luteinizing hormone
PCPT	The Prostate Cancer Prevention Trial
REDUCE	the Reduction by Dutasteride of Prostate Cancer Events
SRD5A1	steroid-5-α-reductase, α polypeptide 1 (3-oxo-5 α-steroid δ 4-dehydrogenase α1)
SRD5A2	steroid-5-α-reductase, α polypeptide 2 (3-oxo-5 α-steroid δ4-dehydrogenase α2)
SRD5A3	steroid 5α-reductase 3

Introduction

Androgens and the androgen receptor (AR) 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. ADT involves the use of GnRH drugs (agonists or antagonists) or orchiectomy, consistently resulting in a 90% to 95% reduction in circulating levels of testosterone. ADTs are quite successful, leading to regression of tumors in a majority of cases; however, selection pressure induced by this treatment ultimately leads to the emergence of a tumor phenotype characterized by disease progression despite castrate levels of testosterone (typically defined as 50 ng/dL, 1.73 nmol/L) , coupled with metastatic spread, which renders the disease lethal. Following 12-18 months, 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 chapter 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.

Androgen Milieu in Blood

Biosynthetic pathway for androgen synthesis is Δ-5 pathway in primates like humans.[6] (Fig. 1) Cytochrome P450s play critical roles in the metabolism of various bioactive compounds. One of the crucial functions of cytochrome P450s in Chordata is in the biosynthesis of steroid hormones. Steroid 17α-hydroxylase/17,20-lyase (CYP17A1) is localized in endoplasmic reticulum membranes of steroidogenic cells. CYP17A1 catalyzes the 17α-hydroxylation reaction of $\Delta4$-C_{21} steroids (progesterone derivatives) and $\Delta5$-C_{21} steroids (pregnenolone derivatives) as well as the 17,20-lyase reaction producing C_{19}-steroids, a key branch point in steroid hormone biosynthesis. Depending on CYP17A1 activity, the steroid hormone biosynthesis pathway is directed to either the formation of mineralocorticoids and glucocorticoids or sex hormones. [7] The first activity gives hydroxylation of pregnenolone and progesterone at the C(17) position to generate 17α-hydroxypregnenolone and 17α-hydroxyprogesterone, while the 17,20-lyase activity cleaves

the C(17)-C(20) bond of 17α-hydroxypregnenolone and 17α-hydroxyprogesterone to form DHEA and androstenedione respectively. The modulation of these two activities occurs through cytochrome b(5). [8] A subset of androgens, adrenal androgens, includes any of C_{19}-steroids synthesized by the adrenal cortex, that function as weak steroids or steroid precursors, including dehydroepiandrosterone (DHEA), its sulfate, DHEA sulfate (DHEA-S), and androstenedione. [9] As a result, a large amount of DHEA and DHEA-S are in the blood in humans and nonhuman primates. The synthesis and secretion of the adrenal androgens DHEA and DHEA-S is a phenomenon apparently unique to humans. DHEA-S is a metabolite of DHEA produced by the addition of a sulfate group, catalyzed by the sulfotransferase. DHEA-S can also be back-converted to DHEA through the action of steroid sulfatase. This layer of the adrenal cortex is thought to be the primary source of serum DHEA-S. DHEA-S levels decline as a person ages as the reticularis layer diminishes in size. In rodents, biosynthetic pathway for androgen synthesis is the Δ-4 pathway.
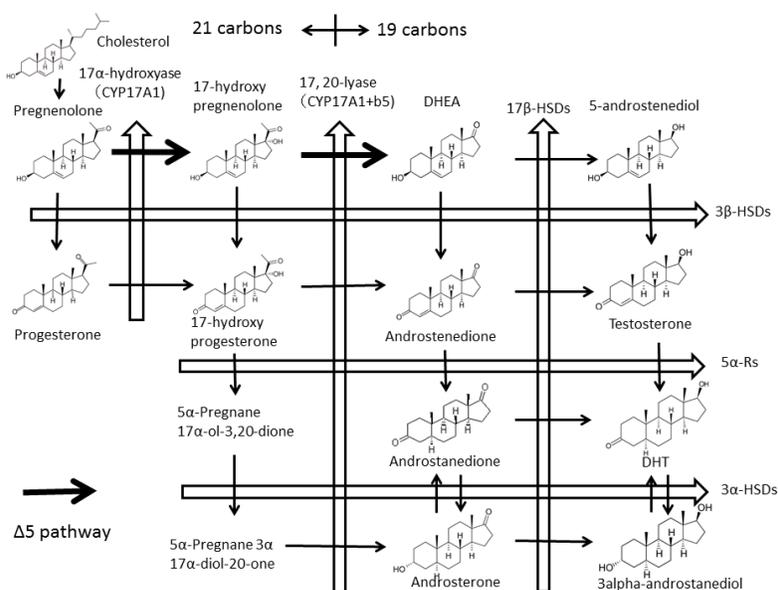
Figure 1. The androgen metabolic pathway and enzyme related products of human being.

Biosynthetic pathway for androgen synthesis is the Δ-5 pathway in primates like humans. As a result, a large amount of DHEA and DHEA-S is in the serum in humans.

b5: cytochrome-b5 reductase

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

DHEA: dehydroepiandrosterone

DHT: dihydrotestosterone

HSD: hydroxysteroid dehydrogenase

5α-Rs: 5-α-reductases.

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. [10, 11] 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. In men, testosterone plays a key role in the development of male reproductive tissues such as the testis and prostate as well as promoting secondary sexual characteristics such as increased muscle, bone mass, and the growth of body hair. [12] In addition, testosterone is essential for health and well-being as well as the prevention of osteoporosis. [13,14] Serum testosterone levels decline with advancing age. [15]

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. [16] Prostate cancer risk and tumour aggressiveness are not related to serum levels of total and free testosterone. [17] 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.

Androgen Milieu in the Prostate

Androgens play an essential role in the development and differentiation of the prostate gland; however, their contribution to pathological conditions, such as benign prostatic hyperplasia and prostate cancer, remains unclear. The main male hormone in circulation is testosterone, which is produced mainly by the testicles. Some male hormones are produced in small amounts by the adrenal glands. However, DHT potently activating AR regulates the prostate. Originating from the gonads, testosterone is released into circulation. Estimates of serum concentrations of total testosterone are unequivocally eugonadal (12.5-40 nmol/L (350-1200 ng/dL)) or hypogonadal (<7.0 nmol/L (200 ng/dL)). [18] 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 17β-hydroxysteroid dehydrogenase (HSD) type 1, 3, 5, 12. [19] 17β-HSD type3 is mainly expressed to convert into testosterone from androstenedione in the testis. Aldo-keto reductase family 1, member C3（AKR1C3）, known as 17β-HSD type5, is mainly expressed to convert into testosterone from androstenedione in the prostate. [20] Testosterone is converted to DHT by steroid-5α-reductase in the prostate. [21, 22] Humans have three isoenzymes of 5α-reductase. Both type 1 and type 2 5α-reductase catalyze the conversion of testosterone to DHT. 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. Testosterone is reduced to DHT by both 5α-reductase type 1 and type 2 where the type 2 enzyme plays the more dominant role in the prostate [23]. Recently, the existence of 5α-reductase type 3 (encoded by SRD5A3) is identified. [24] Godoy et al. showed that expression of 5α-reductase type 3 in "classical" as well as "non-classical"

androgen-regulated tissues is consistent with 5α-reductase type 3 enzyme having functions other than converting testosterone to DHT in human tissues, such as participation in the N-glycosylation process.[25] They also showed that over-expression of 5α-reductase type 3 in breast, testis, lung, thyroid, and particularly prostate cancer, compared to their benign counterparts, suggests a potential role for 5α-reductase type 3 as a biomarker of malignancy, and over-expression of 5α-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. DHT is reduced by 3α-HSD type 3 (AKR1C2) to produce 3α-diol and by 3β(20α)-HSD (AKR1C1 (98 % sequence identity with AKR1C2)) to produce 3β-diol. [26, 27] AKR1C2 is the dominant 3-ketosteroid reductase that will convert DHT to 3α-diol and will prevent DHT from binding to its receptor. AKR1C1 instead converts DHT to 3β-diol, where 3β-diol is a pro-apototic ligand for estrogen receptor β [28,29].

Recently, DHT mainly synthesizes via testosterone as stated above, but also synthesizes without testosterone biosynthesis, 'backdoor' pathway. [22, 30, 31] 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-α-reductase type 1 to convert 17-hydroxyprogesterone to 5-α-reduced androgen precursors.[32] In this pathway progesterone is converted to 17α-hydroxyprogesterone by CYP17α-hydroxylase/17,20 lyase and then reduced by SRD5A1 to yield 5α-pregnane-17α-ol-3,20-dione. This is then reduced by AKR1C2 to yield 5α-pregnane-3α,17α-diol-20-one. Subsequent reaction by CYP17α-hydroxylase/17,20-lyase would produce androsterone which is then reduced by AKR1C3 to 3α-diol. Thus a new molecular switch that controls ligand access to the AR has been identified as AKR1C2 which reduces DHT to 3α-diol and oxidative 3α-HSDs which oxidizes 3α-diol back to DHT (in stromal cells). [33] Five different short-chain dehydrogenase genes (HSD17B10, HSD17B6, RDH5, DHRS9, RODH4) have been invoked as being the oxidative 3α-HSDs responsible for this back conversion. However, detailed kinetic analysis using mammalian expression systems and expression profiling have provided unequivocal evidence that the enzyme responsible is "RoDH like 3α-HSD" (HSD17B6) [34]. 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.[35, 36]   Studies have failed to show increased risk of prostate cancer in men with higher serum testosterone, and supraphysiologic testosterone fails to increase prostate volume or prostate-specific antigen in healthy men. This apparent paradox is explained by the Saturation Model, which posits a finite capacity of androgen to stimulate prostate cancer growth. 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. Modern studies indicate no increased risk of prostate cancer among men with serum testosterone in the therapeutic range. [37, 38]

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. A androgen milieu may be associated with tumor aggressiveness; however, 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.[16, 39] Prostate cancer risk and tumor aggressiveness are not related to serum levels of total and free testosterone. [40] 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. [41] The plasma concentration of DHEA-S is 100-500 times higher than that of testosterone. [42] 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.[42] 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. [43] 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. [44] Several studies suggested that men with smaller prostates had more high-grade cancers and more advanced diseases. [45-47] 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-α-reductase inhibitor, for the prevention of carcinoma of the prostate was held. [48] 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. [49] 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. [50] Moreover, the results of the Reduction by Dutasteride of Prostate Cancer Events (REDUCE) trial using the dual 5-α-reductase isoenzyme inhibitor dutasteride, has recently been reported. [51] 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.

Both AR and androgen-converting enzymes have been reported to be up-regulated in high-grade or advanced prostate cancer. [52-54] AR is a ligand-inducible transcription factor, a member of the nuclear receptor superfamily, which plays an important role in the development and progression of prostate cancer. High levels of 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. [52] This confirms the role of AR in tumor growth and progression in hormonally naive prostate cancer. Several reports and our results revealed that 17β-HSD5 (known as AKR1C3) positive cases were significantly associated with clinical stage. [53, 54] Fung et al. revealed that elevated expression of AKR1C3 is highly associated with prostate cancer. [53] We also revealed that both AR and androgen-converting enzymes were up-regulated in the high-grade or advanced prostate cancer. [54] AKR1C3 (17β-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.

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 (1.73 nmol/L). [55-58] In men with prostate cancer,

orchiectomy reduces serum testosterone to anorchid levels within 12 hours. GnRH agonists have played an important role in preventing progression of prostate cancer by lowering testosterone levels. However, initial use of agonists causes testosterone to surge while repeated doses cause microsurges. [59] Moreover, breakthrough increases over castrate levels increase over time. [60] 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. [61] 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 below 20 ng/dL (0.7 nmol/L). Morote et al. have shown that 25 percent of men receiving continuous ADT with a GnRH agonist have a serum testosterone level greater than 50 ng/dl.[62] Forty-four percent of the same group of men studied had a serum testosterone level that was consistently below 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. [63] Zaitsu et al. showed that even hormone refractory prostate cancer patients are candidates for surgical castration because of endocrinological, oncological, and economical reasons. [64] Testosterone levels should be monitored routinely in patients on GnRH agonists so that the treatment can be switched or an antiandrogen agent can be added when there is a breakthrough. [65]

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. [42] Previous reports revealed that the mean DHT levels in the prostate tissue treated with ADT were between 10% and 40% of those of untreated. [42, 66-70] Testosterone is converted to DHT by 5-α 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. [67] 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. [42] 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. [71] Recently, 5α-reductase type 1(SRD5A1) is reported to express in aggressive and recurrent prostate cancer. [54,72, 73] Arai et al. showed that the levels of adrenal androgens in prostate cancer tissues after ADT were similar to those in untreated prostate cancer. [74] 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. [75] 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. [76] 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. [77] 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. [78] The androgen milieu in men with high Gleason score prostate cancer is probably less affected by conventional ADT than that in men with low Gleason 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 adrenocorticotropic hormone has a central role in the regulation of androgen synthesis.[79] Serum adrenocorticotropic hormone and adrenal androgen concentrations were correlated with the post-treatment PSA. Adrenocorticotropic hormone mediated androgen synthesis is a potential target for advanced androgen deprivation therapy.

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.[80] 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.[81] 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. [82] 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. [83, 84] 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. [85] Although the pathway from adrenal precursor steroids to intratumoral synthesis of DHT was widely believed to require testosterone, the main route instead circumvents testosterone through the 5α-androstanedione pathway and requires expression of SRD5A1. Strategies for the development of better therapeutic approaches should account for the unanticipated dominance of the 5α-androstanedione pathway in CRPC. [86] Moreover, AR transactivation in prostate cancer cells in the presence of androstanediol resulted from the cell-specific conversion of androstanediol to DHT. [87] 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. [88, 89] 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. [89] 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. [90] 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. [91] 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. [92] 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.

Clinical-Translational Advances of Prostate Cancer

The hypothesized dependence of prostate cancer on androgen stimulation for survival and proliferation even in the CRPC state corroborate the rationale for drug development strategies that target androgenic ligands as well as the AR itself. The promising agents of therapies that directly target androgen synthesis as well as the AR confirm that prostate cancer retains dependence on AR signaling, even in the CRPC state. 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 and CRPC have been reported.

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. [93] The more recently developed GnRH antagonists act immediately. 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. [94] 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 gonadotropin-releasing hormone antagonist for prostate cancer, has been linked with immediate-onset systemic allergic reactions. [95] Degarelix is a new type of hormonal therapy for prostate cancer without stimulation of the release of histamine. [96] 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. [97] 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 androgen deprivation therapy was indicated. [98] 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 (<or=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. [99] As with all hormonal therapies, degarelix is commonly associated with hormonal side effects such as hot flushes and weight gain. [98] 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.

The GnRH agonists initially stimulate before suppressing testosterone and follicle-stimulating hormone (FSH) production. FSH is speculated to play a role in prostate cancer progression. [100] Compared to normal prostate cells, cancer cells contain more FSH receptors and more so in androgen refractory prostate cancer cells. FSH receptor is selectively expressed on the surface of the blood vessels of a wide range of tumors. [101]

Cytochrome P450, Family 17, Subfamily A, Polypeptide 1 (CYP17A1) Inhibitors

The multifunctional 17α-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 17α-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. [102, 103] 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 . [104, 105] 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. [106, 107] Additionally, some evidence suggests that CYP17A1 mRNA and protein expression correlate with disease stage and relapse. [108,109] Despite the strong rational 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.

Abiraterone Acetate (Zytiga, Johnson & Johnson)

One of CYP17A1 inhibitors, abiraterone acetate, the irreversible inhibitor of 17α-hydroxylase/C17,20-lyase (CYP17A1), is well tolerated and has significant and durable anti-tumor activity both in patients with chemotherapy-naive prostate cancer and in docetaxel-treated patients with CRPC. [110] 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. [111] 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. [112-114] 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). [115] 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, CRPC 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. 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. 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). Herein, several potential strategies for abiraterone acetate are presented to clarify the clinical utilization of this agent in the future.

Abiraterone acetate is a prodrug of abiraterone. Most notably, it has a food effect greater than any other marketed drug (five- to 10-fold, depending on fat content), yet it is labeled to be taken fasting. The marked effect of a high-fat meal (10-fold increase in exposure) implies that absolute bioavailability in the fasting state is no more than 10%. This is likely the result of low absorption under fasting conditions, because 77% of the administered drug is excreted as abiraterone (or its prodrug acetate) in feces. In addition, there is marked interindividual pharmacokinetic variability, with a coefficient of variation in the plasma concentration area under the curve of 59% under labeled conditions. [116] Food can alter the bioavailability of orally administered drugs. Description of food effects in product labels and information about administration in relation to food are influenced by a variety of factors. Because food effects can change drug efficacy and toxicity, it is important that physicians and patients be aware of them. [117, 118]

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 (IC50 = 28nM) currently in clinical trials in prostate cancer patients. The compound was found to be selective for CYP17A1 over 11β-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. [119, 120] 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 >= 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.

TOK-001 (VN/124-1) [3β-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. [121, 122]. 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) phase1/2 trials with TOK-001 was recently initiated in CRPC patients.

3β-Hydroxysteroid Dehydrogenase Inhibitors

Prostate cancer exhibits robust 3β-HSD enzymatic activity, which is required for the AR-mediated response to Δ-5 adrenal androgens and that 3β-HSD may serve as a pharmacologic target for the treatment of CRPC. [123] Pharmacologic inhibition of 3β-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 3β-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.[124] 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 3β-HSD free of AR agonist activity.

Apoptone (HE3235) (17α-Ethynyl-5α-Androstan-3α, 17β-Diol)

A novel androstane steroid, Apoptone (HE3235), an orally bioavailable synthetic analogue of 3β-androstanediol, has a significant inhibitory activity for 5'-androstenediol-stimulated LNCaP proliferation. [125, 126] 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 tumors. 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. [127]

Inhibitors Targeting Androgen Receptor

A New Androgen Receptor Antagonist, MDV3100

As for the conventional anti-androgen, affinity for AR and agonistic activity for AR are matters of concern. [82] 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 diarylthiohydantoins) is a small-molecule, pure AR antagonist that inhibits AR nuclear translocation and DNA binding. [128, 129] 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-5α-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 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). The company, Medivation, announced on November 3, 2011, that MDV3100 improved survival by 4.8 months, compared with placebo, in the AFFIRM trial. The AFFIRM trial is a randomized, double-blind, multinational trial comparing MDV3100 (160 mg/day) with placebo in 1199 men with advanced prostate cancer who were previously treated with docetaxel-based chemotherapy. Enrollment was completed in November 2010, and the interim analysis was triggered at 520 events. A planned interim analysis of the AFFIRM trial revealed that estimated median survival was 18.4 months for men treated with MDV3100, compared with 13.6 months for men treated with placebo (P <0.0001). This was

construed that MDV3100 improved survival by 4.8 months compared with placebo. This translates into a 37% reduction in the risk for death with MDV3100 (hazard ratio, 0.631). As a result, the trial's Independent Data Monitoring Committee recommended that AFFIRM be stopped early and that men who were receiving placebo be offered MDV3100.

A New Androgen Receptor Antagonist, ARN-509

A new androgen receptor antagonist, 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)

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. [130] 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.

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 Gleason score cancer, which was suggested to be associated with adrenal androgen levels. In patients treated with ADT the pituitary-adrenal axis mediated by adrenocorticotropic hormone has a central role in the regulation of androgen synthesis. Adrenocorticotropic 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.

Conclusion

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 Gleason score cancer, which was suggested to be associated with adrenal androgen levels. In patients treated with ADT the pituitary-adrenal axis mediated by adrenocorticotropic 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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