Asian Journal of Andrology (2017) 19, 143–148

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REVIEW

Prostate Cancer

Understanding prostate-specific antigen dynamics in monitoring metastatic castration-resistant prostate cancer: implications for clinical practice

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Availability of novel hormonal therapies as well as docetaxel and cabazitaxel treatment for metastatic castration-resistant prostate cancer (CRPC) has changed the outlook for this group of patients with improvements in progression-free survival and overall survival. Physicians often diagnose the progression of prostate cancer using serum prostate-specific antigen (PSA). However, serum PSA is not always correlated with the clinical status in CRPC. To evaluate the PSA dynamics with greater precision, understanding of the control of PSA and of the mechanisms of development of CRPC is needed. Moreover, it is necessary to use new hormonal therapies with an appropriate timing to optimally improve the prognosis and the QOL of the patients. In the present review, we ascertain the PSA dynamics and the mechanisms of the development of CRPC to assist in optimal utilization of the new treatments for mCRPC.
Asian Journal of Andrology (2017) **19**, 143–148; doi: 10.4103/1008-682X.179159; published online: 3 June 2016

Keywords: castration-resistant prostate cancer; new hormonal therapy; prostate-specific antigen

INTRODUCTION

Androgen deprivation therapy (ADT) is generally the first choice for the management of advanced prostate cancer. Unfortunately, after an initial response to ADT, prostate cancer eventually loses responsiveness to the androgen blockade and progresses into castration-resistant prostate cancer (CRPC). Physicians often use prostate-specific antigen (PSA) as a biomarker to diagnose and follow-up prostate cancer. Especially, elevation of serum PSA during ADT contributes to the early diagnosis of CRPC. The serum PSA value, however, does not always correlates with the malignant state of CRPC. In some cases, there can be worsening of symptoms though the PSA values are low and stable in that patient.

Recently, new hormonal therapy agents, abiraterone acetate and enzalutamide, which block androgen receptor (AR) axis signal pathway are available for the treatment of CRPC. These medicines eventually improve the prognosis of patients with CRPC. However, the optimal timing for use of these novel hormonal therapies is still not clearly established. Therefore, physicians need to understand the mechanisms by which progression of prostate cancer may affect the serum PSA and subsequent development of CRPC.

In this review article, we describe the implications of changes in PSA dynamics and mechanisms of development of CRPC. We also discuss on optimizing treatment strategies in the use of classical hormonal therapies and new hormonal therapies.

WHAT IS THE MECHANISM OF SERUM PSA CHANGES?

PSA was first purified from prostate and seminal plasma. Wang *et al.*[1] examined the serum PSA in patients with prostate cancer, since PSA was localized within prostatic ductal epithelial cells and was secreted into the medium where prostate cancer-derived cell lines were cultured.[2,3] They found that serum PSA value decreased in respond to the treatment and increased on recurrence of prostate cancer. Now, PSA has been widely used for the diagnosis of prostate cancer and for monitoring patients with prostate cancer. PSA is especially available as a good tool to diagnose the progression of prostate cancer after androgen deprivation therapy (ADT) or chemotherapy. PSA (KLK3), one of the kallikrein-related peptidases (KLKs) belonging to a family of proteases, is associated with sperm motility by changing semen liquefaction.[4–6] In cancer, PSA may induce the adhesion of prostate cancer cells to bone marrow cells and proliferation of osteoblasts.[7,8]

The expression of PSA mRNA is usually stimulated by androgens.[9] This regulation is mainly mediated through the PSA promoter that contains at least three androgen-response elements (AREs)[10,11] (**Figure 1**). Especially, the 4.1 kb upstream region of the transcription initiation site of PSA mRNA is extremely important for the androgens to induce PSA promoter activity.[11,12] When androgen receptor (AR) with DHT binds to AREs of the PSA promoter, the expression of PSA mRNA is up-regulated. Conversely, the PSA mRNA expression in the prostate is down-regulated by ADT, and the serum PSA protein level is also decreased. PSA itself, however, is not a growth factor that stimulates the proliferation of prostate cancer directly. The proliferation of LNCaP-SF cells in charcoal-stripped fetal calf serum instead of fetal calf serum from androgen-sensitive LNCaP cells was repressed by DHT, although the expression of PSA mRNA was simultaneously induced by DHT in a dose-dependent manner,

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Received: 06 August 2015; Revised: 11 October 2015; Accepted: 01 March 2016

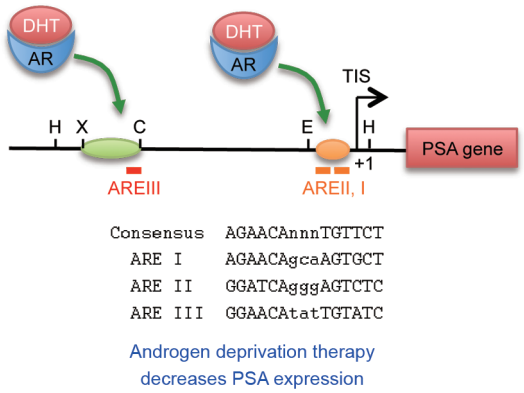

Figure 1: Primary structure of the PSA promoter. PSA mRNA is transcribed from the transcription initiation site (TIS) (+1). E: EcoRI, C: ClaI, X: XbaI, and H: Hind III restriction enzyme site. The consensus sequence of androgen response element (ARE) is AGAACAnnnTGTTCT.

suggesting that the proliferation of prostate cancer cells is not always correlated with the expression of PSA.[13]

Postulated mechanisms that explain why serum PSA is elevated in prostate cancer before ADT are (1) Because basal cells disappear in prostate cancer, and the ductal structure is destroyed, PSA can leak easily into the blood stream from a duct[14,15] (2) AR activity can also be elevated by some mechanisms, such as AR amplification, increased AR protein, or involvement of various AR coactivators[16–18] (3) On a tumor volume effect with an increased number of prostate cancer cells, serum PSA is elevated as cells secrete a slight dose of PSA. For example, if patients with prostate cancer have many bone metastases, the serum PSA level should increase. However, the serum PSA levels are considerably different among patients even when the levels of bone metastases and local prostate volume are practically similar.

MECHANISMS OF PSA DECREASE AND INCREASE DURING HORMONAL THERAPY

As described above, PSA expression from one prostate cancer cell is basically regulated by the androgens' axis, and the serum PSA level is regulated not only by androgen, but also by tumor volume. **Figure 2** shows one typical clinical case in which prostate cancer with bone metastasis was treated with ADT.

This case illustrates the mechanism of PSA decrease and increase during ADT. The initial serum PSA was 2163 ng ml^{-1}, Gleason score was 4 + 4 = 8, and Stage was T4, N1, and M1b. When the bone metastasis extent was measured using the EXINI bone (BONENAVI) computer-assisted diagnosis (CAD) system,[19,20] the bone scan index (BSI) was 3.9% (**Figure 2a**). After the patient had been treated with LH-RH agonist and bicalutamide as initial ADT, the serum PSA level decreased to 12.1 ng ml^{-1} rapidly in 3 months. The half-life of serum PSA in this period was approximately 12 days (**Figure 2b**). Most likely, the initial rapid decrease of serum PSA was a result from the inactivation of PSA promoter by ADT (PSA-androgen response phase in **Figure 2c**). Then, the serum PSA gradually decreased to a nadir 3.1 ng ml^{-1} in the next 6 months. The half-life of serum PSA in this period was approximately 90 days (**Figure 2b**). The slow decrease of the serum PSA could be resulted from a decrease of the prostate cancer cell number by apoptosis through several signal pathways induced by ADT (tumor regression phase in **Figure 2c**). After this phase, the decrease in the serum PSA stopped and reached nadir (3.1 ng ml^{-1}). BSI also decreased to 0.12% (**Figure 2b**). In this situation, the ratio of proliferating cells and dying cells is potentially balanced (proliferation quiescent phase in **Figure 2c**). Finally, prostate cancer cell proliferation that was regulated by ADT relapsed became castration-resistant prostate cancer (CRPC). As a follow-up, serum PSA increased to 41.8 ng ml^{-1} after 8 months of the PSA nadir. BSI was also elevated to 4.3% after 10 months of PSA nadir. Although serum PSA was still low (approximately 2% compared with the initial serum PSA value), BSI in this situation was almost at the same value as at initial diagnosis. What does this mean? Several mechanisms of progression into CRPC have been proposed, such as AR mutation, adaptation to low androgen environment, and clonal selection.[21–26] A great deal of interest has been paid to the clonal selection of androgen-independent prostate cancer cells in these mechanisms. When AR expression in prostate cancer tissue is investigated by immunohistochemistry, AR expression is often heterogeneous in cancer patients.[27] AR expression was significantly correlated with the endocrine response, time to progression, and survival ($P = 0.02$).[17,28] AR content in prostate tumor cells also became more variable with an increasing Gleason score.[29] As described above, tumor volume effect caused by AR-negative prostate cancer cells (androgen-independent prostate cancer cells) at the bone metastasis site might raise serum PSA in this CRPC case (**Figure 2c**). Therefore, the severity in bone metastases in CRPC can be the same as pretreatment even if the serum PSA on CRPC is at a low value, since growth factors in bone can replace androgens as stimulators of cancer cell proliferation.

As described in this case, sometimes, the degree of bone metastases does not coincide with an absolute value of serum PSA. Clinicians, therefore, need to examine not only serum PSA level, but also bone scintigraphy regularly when they follow-up CRPC patients with bone metastasis.

LEVEL OF PSA NADIR AND PROGNOSIS

It is generally accepted that the scenario of a patient having high Gleason score in spite of low serum PSA level at initial diagnosis is often associated with a poor prognosis compared with a low Gleason score. In such cases, the level of AR expression might be low or the ratio of AR-positive cells might be low in the prostate cancer tissue. In this situation, the serum PSA level can be low because PSA is mainly regulated by androgen as described above.

Clinicians also recognize that the prognosis of patients with a high PSA nadir after ADT is generally poor.[30,31] There is no clear evidence to explain the mechanisms of this observation. We hypothesize that the presence of androgen-independent prostate cancer cells before ADT could explain this observation (**Figure 3**). When the majority of prostate cancer cells are androgen-sensitive, and androgen-independent cells hardly exist before ADT, decline of serum PSA is dependent on ADT and on apoptosis by ADT (**Figure 3a**). Moreover, the cases that take long time until a serum PSA level reaches a nadir and a serum PSA is elevated again during ADT have better prognosis.[31] This reason is that a ratio of androgen-independent cells is low before ADT, and it takes long time for androgen-sensitive prostate cancer cells to adapt themselves to low androgen environment. In contrast, when a lot of androgen-independent cells exist and the ratio of these cells is high before ADT, ADT cannot diminish serum PSA level dramatically and PSA nadir is high because the serum PSA level is dependent on the total number of androgen-independent prostate cancer cells as described above (**Figure 3b**). In such cases, the effectiveness of second-line anti-androgen may not be so promising due to the preponderance of androgen-independent prostate cancer cells.

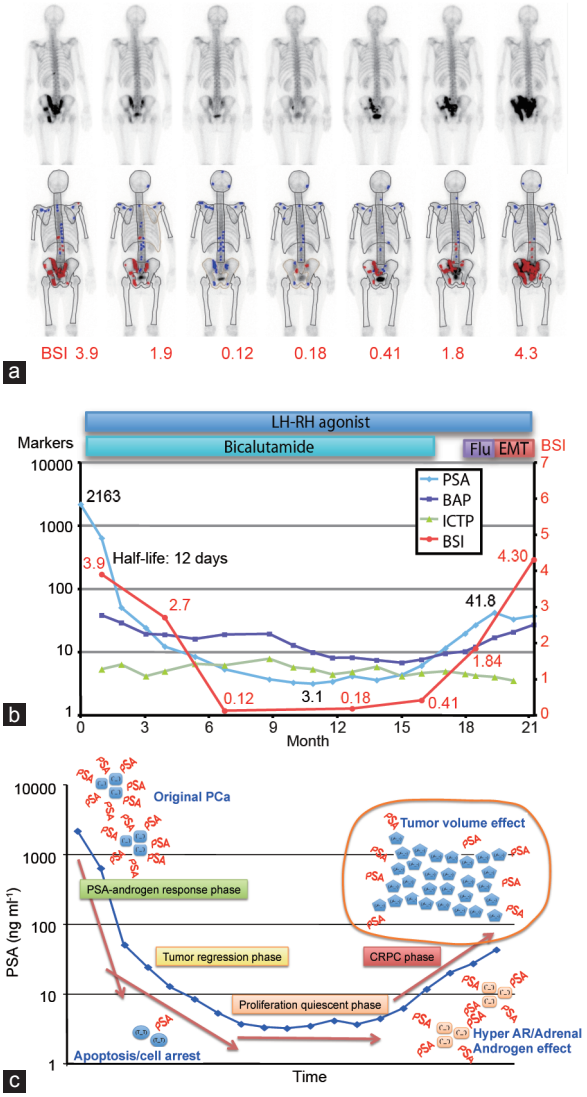

Figure 2: Change of bone metastasis volume and PSA levels by ADT. (**a**) Bone scan index (BSI) was measured by EXINI bone (BONENAVI). (**b**) Changes in PSA, bone metabolic markers (BAP and I-CTP), and BSI by hormonal therapy. Flu: flutamide and EMP: estramustine phosphate. (**c**) Change of PSA and mechanism of PSA rising during ADT.

CHAARTED trial (Androgen ablation therapy with or without chemotherapy in treating patients with metastatic prostate cancer) and STAMPEDE trial (Docetaxel and/or zoledronic acid for hormone-naive prostate cancer: first overall survival results from STAMPEDE) showed that addition of six courses of docetaxel chemotherapy to initial hormonal therapy improved overall survival in patients with hormone-naive metastatic prostate cancer.[32,33] These results indicate that androgen-independent prostate cancer cells present in high volume metastatic prostate cancer before ADT may be potentially killed by initial docetaxel treatment (**Figure 3c**).

NEW HORMONAL THERAPIES

Recently, new hormonal agents have been available for CRPC treatment, one being enzalutamide, a second-generation anti-androgen while the other one is abiraterone acetate, an adrenal androgen synthesis inhibitor. When we look at the effectiveness of these new agents, we

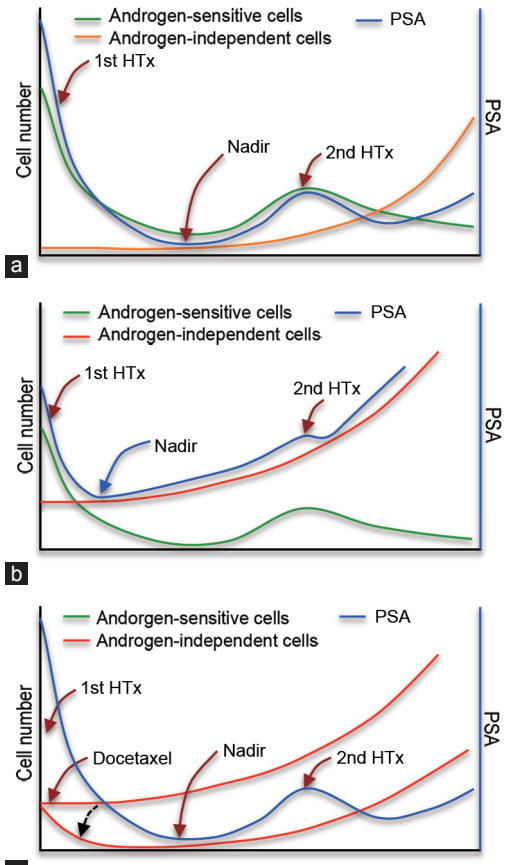

Figure 3: Potential explanation for the prognosis of a patient where the PSA nadir is high and time to PSA nadir is short is generally poor. (**a**) Situation with few androgen-independent cells before ADT. (**b**) Situation with many androgen-independent cells before ADT. (**c**) Docetaxel decreases the number of androgen-independent prostate cancer cells.

notice a difference in responsiveness for these new agents between overseas and Japan. In the AFFIRM trial (phase 3, double-blind, placebo-controlled trial, 1199 men with CRPC after chemotherapy), the major clinical efficacy end points, overall survival (OS), and time to PSA progression (TTPP) were evaluated.[34] Enzalutamide consistently improved OS and TTPP compared with placebo. The median OS was 18.4 months (95% confidence interval [95% CI], 17.3 to not yet reached) in the enzalutamide group versus 13.6 months (95% CI, 11.3–15.8) in the placebo group, and TTPP was 8.3 in the enzalutamide group versus 2.9 months in the placebo group (hazard ratio, 0.40; $P < 0.001$). In Japan, a single arm phase 1/2 study was conducted. The median OS and TTPP were 10.6 months (95% CI, 6.9 to not yet reached) and 4.1 months (95% CI, 2.9–6.6) after chemotherapy in the enzalutamide group, respectively. In Japan, enzalutamide did not improved OS and TTPP like in the AFFIRM trial. A phenomenon similar to the clinical studies with enzalutamide was observed in clinical studies with abiraterone acetate. The COU-AA-301 randomized, double-blind, placebo-controlled phase 3 study showed that median OS for the abiraterone group was longer than in the placebo group (15.8 months [95% CI, 14.8–17.0] *vs* 11.2 months [10.4–13.1]; 95% CI, 0.64–0.86; $P < 0.0001$). Median TTPP was also improved in the abiraterone group (8.5 months, 95% CI, 8.3–11.1, *vs* 6.6 months, 5.6–8.3, in the placebo group).[35] In a similar study conducted in Japan (JNJ-212082-JPN-202), median TTPP in

JNJ-212082-JPN-202 study in the abiraterone acetate group was shorter than overseas (3.6 months [2.8–3.8]).[36]

We speculate that the reason why the overseas results were better than the Japanese ones in both the enzalutamide and abiraterone acetate studies is mainly due to a difference of the number of anti-androgens used before chemotherapy (**Figure 4**). In the AFFIRM study, 338/392 (86.2%) of patients were treated only with 0, first-line, or second-line hormonal therapy before enrollment. In contrast, all patients were treated with second-, third- or fourth-line hormonal therapy before enrollment in Japan. In the COU-AA-301 study, 18%, 65%, and 15% of registered patients were treated with 0, 1, and 2 anti-androgens, respectively.[37] In contrast, 0%, 12.8%, 48.9%, 23.4%, 10.6%, and 4.3% of enrolled patients were treated with 0, 1, 2, 3, 4, and 5 anti-androgens in Japan, respectively.[36] The AFFIRM and COU-AA-301 studies revealed that docetaxel is eventually not sufficient to attack androgen-hypersensitive prostate cancer cells. However, sequential classical hormonal therapies including anti-androgens (bicalutamide, flutamide, chlormadinone acetate, and estrogens) in Japan might regulate androgen-hypersensitive prostate cancer cells (**Figure 5**).

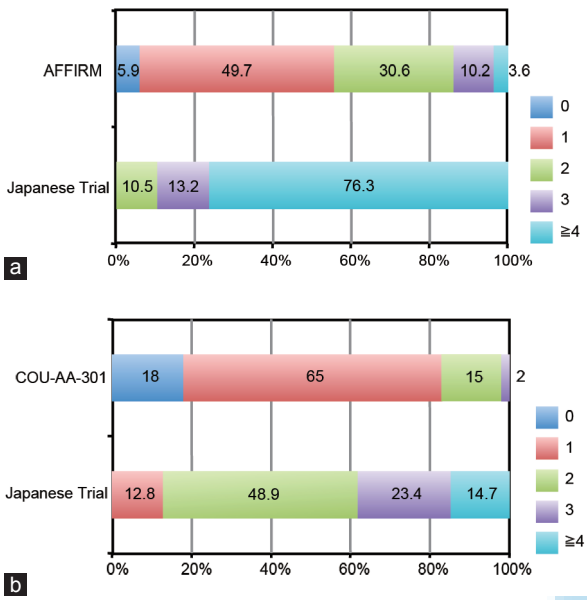

Figure 4: Number of anti-androgens before docetaxel treatment. (**a**) Clinical trial of abiraterone acetate after docetaxel. (**b**) Clinical trial of enzalutamide after docetaxel.

Another reason for the enzalutamide and abiraterone studies in Japan showing inferior results to the pivotal registration trials overseas could be due to difference of the use of docetaxel in Japan. Although docetaxel can be used only up to ten courses irrespective of the effectiveness in overseas, in Japan, it is used for as many courses as required without limitation as long as docetaxel is effective and is being tolerated. Use of docetaxel for a long time (more than ten courses) may inhibit the growth of androgen-hypersensitive prostate cancer cells. It is possible that the patients who were registered in the trials were treated with docetaxel for a long time. When CRPC relapses again after docetaxel treatment in Japan, it is very likely that CRPC has already lost androgen sensitivity. As a result of these reasons, enzalutamide and abiraterone acetate may not be so effective after long-term docetaxel treatment any more in Japan.

These causes may reflect the relative inefficacy of novel hormonal therapies after docetaxel treatment in Japan. Therefore, clinicians need to consider the total duration of docetaxel treatment and prior hormonal therapies to optimize the use of novel hormonal therapies.

FUTURE TREATMENT STRATEGY FOR CRPC

Clinicians should confirm the PSA nadir level and time to PSA nadir following primary ADT. These surrogate markers would predict the prognosis of CRPC.[38] The previous duration of response to ADT may also become a predictor of sensitivity to next generation AR axis-targeted drugs in patients with mCRPC.[31] However, there is no clear evidence about the optimal sequencing strategy to achieve the best prognosis, namely the new hormonal therapies (enzalutamide or abiraterone acetate) or docetaxel treatment.

Based on the mechanism of PSA elevation described above, clinicians can postulate which cell population contributes to the progression of

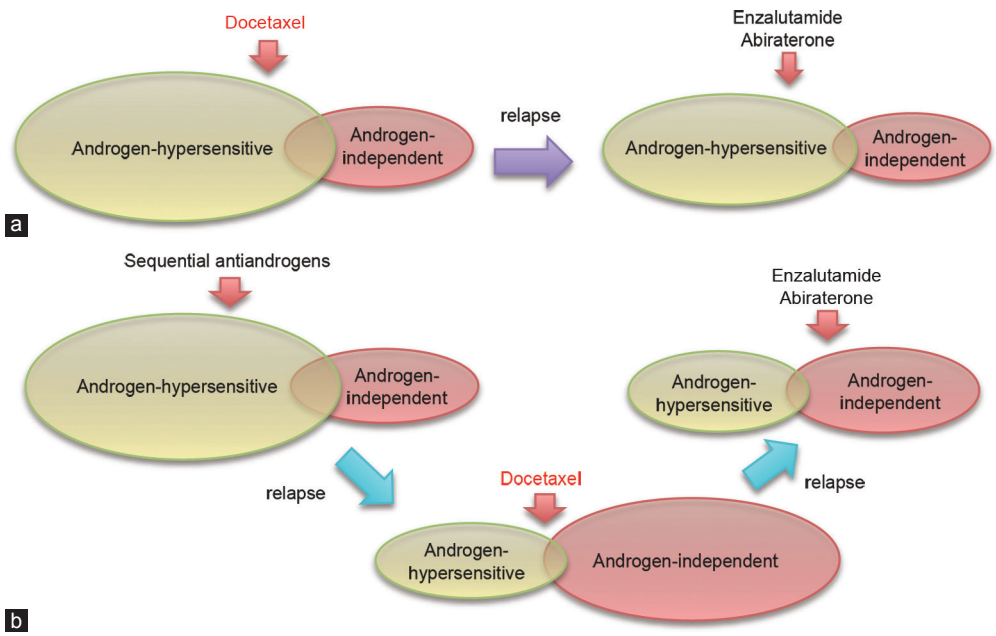

Figure 5: Difference of cell population between overseas patients and Japanese patients when new hormonal therapies were used after docetaxel treatment for metastatic disease. The situation of the cell population at the time of the clinical study (abiraterone and enzalutamide) performed abroad (**a**) and Japan (**b**).

prostate cancer after classical hormonal therapy. To help distinguish the predominant cell population in CRPC after classical hormonal therapy, the use of classical second-line anti-androgen therapy may be one alternative. When classical second-line anti-androgen therapy is not effective, it can be considered that an androgen-independent cell population has become predominant and the AR axis plays a minor role in the progression of disease. In this case, docetaxel could be used first before using new hormonal therapy. In contrast, it can be hypothesized that the AR axis is clearly involved in recurrence when anti-androgen reduces serum PSA level.[21,39] The prognosis of such patients is expected to be better.[40] Considerably, an androgen-sensitive cell population is predominant in such a case. Then, the way can be opened to conduct other third-line or fourth-line hormonal therapy.

New hormonal therapies using enzalutamide and/or abiraterone acetate can significantly decrease the risk of radiographic progression and death and delay the initiation of chemotherapy in men with metastatic prostate cancer.[41,42] However, it does not mean that classical hormonal therapies used until recently are not useful following the availability of enzalutamide and abiraterone acetate. Not only anti-androgen, but also estrogen agents (ethinyl estradiol and diethylstilbestrol) have been effective for CRPC.[43,44] Omlin *et al.* reported that abiraterone acetate had antitumor activity in men with CRPC even after diethylstilbestrol.[45] If this retrospective observation is reproducible, abiraterone acetate could be used after estrogen agents, since an additional PSA response by diethylstilboestrol could be expected before abiraterone acetate treatment. Moreover, the early use of the new hormonal medicines could also induce resistance to these medicines early.

Several groups have already described the potential mechanisms of resistance for enzalutamide and abiraterone acetate. Cells expressing the AR F876L mutation confer an antagonist-to-agonist switch that drives phenotypic resistance to enzalutamide.[46] Rodriguez-Vida *et al.*[47] reported that an enzalutamide withdrawal effect was observed in 10% (3/30) of patients with metastatic CRPC after docetaxel chemotherapy. Moreover, although the CYP17A1 inhibitor abiraterone acetate markedly reduces androgen precursors and is thereby effective in CRPC, abiraterone increases progesterone, which may be selected for progesterone-responsive mutant AR, such as T877A. This selection may activate androgen-responsive genes.[48]

Furthermore, AR splice variants may also confer resistance to enzalutamide and abiraterone.[49] AR splice variant (AR3 or AR-V7) is up-regulated during prostate cancer progression and promotes androgen depletion-resistant growth.[50–53] Liu *et al.*[54] have described a molecular mechanism of AR splicing. Under ADT conditions, recruitment of several RNA splicing factors to the 3'-splicing site for AR-V7 (U2AF65 and ASF/SF2) was increased. Activation of U2AF65 and ASF/SF2 expression by strong ADT using enzalutamide and abiraterone might further induce AR splicing. AR-V7 induced by ADT modulates not only canonical androgen-responsive genes, such as PSA, TMPRSS2, NKX3.1, but also AR-V7-specific genes, such as UBE2C, ACOX1, MAP2K4, IGFBP3, NRP1, and multiple tumor-promoting autocrine/paracrine factors.[55–57] Especially, ACOX1 and MAP2K4, and a high expression of IGFBP3 and NRP1 were significantly associated with shorter time to PSA failure.[57] AR-V7 overexpression may also cause docetaxel-resistance.[58] Therefore, attention should be paid to the possibility that maximizing ADT in the early phase of CRPC might cause resistance to other treatments.

CONCLUSION

Monitoring for metastatic CRPC with PSA measurements alone is not adequate. Therefore, we need to evaluate bone metastasis by bone scintigraphy and quantification of bone metastasis as well as other imaging studies. Moreover, from the results of CHAARTED trial and STAMPEDE trial, positioning of chemotherapy for the prostate cancer with high risk and high volume metastasis is changing now.[32,33] As clinicians are likely to use docetaxel upfront with ADT in metastatic prostate cancer, this may have an impact on the timing and nature of metastatic CRPC (mCRPC). Despite the trial results of enzalutamide and abiraterone in the predocetaxel setting in mCRPC, the robustness of an overall survival benefit in relation to these drugs given in the postdocetaxel setting remains uncertain, especially as there has been no Phase 3 RCT to address this question. Sequential therapy using these medicines after classical anti-androgens and other proven therapies may further prolong the life of patients better than single therapy. Clinicians should evaluate various factors including prognosis, QOL, and cost-effectiveness along with patient preference when considering which treatments will be most beneficial for the patients. However, the optimal sequencing strategy needs to be robustly evaluated in clinical trials.

AUTHOR CONTRIBUTION

AM is the corresponding author and managed everything. KI, HK, YK, YK, KN, TN, and MN contributed to the discussion about CRPC and drafted the manuscript. AKB contributed to the discussion about CRPC and corrected English grammar.

COMPETING INTERESTS

All authors declare no competing interests.

ACKNOWLEDGMENTS

We appreciate Prof. Fernand Labrie for his valuable discussion.

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