

REVIEW ARTICLE

The androgen receptor remains a key player in metastatic hormone-refractory prostate cancer. Implications for new treatments

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Summary

Metastatic castration-resistant prostate cancer (mCRPC) shows a number of adaptive mechanisms that facilitate continued androgen receptor (AR) dependent tumor growth. In this article we reviewed the subsequent hormonal manipulation in mCRPC, including the recently approved new drugs, in relation to the AR dependent and independent growth mechanisms. Maintaining castrate levels of testosterone is mandatory. The AR amplification, a process that can occur within the hypersensitive AR escape route, can be fought by using high dose antiandrogen (bicalutamide 150mg), change in antiandrogen preparation or the use of enzalutamide. Switch to another antiandrogen, the use of LHRH antagonists, change to another LHRH agonist, bilateral orchidectomy, adrenals' inhibition and the blockade of intratumor testosterone synthesis are several ways to counter the increased AR sensitivity. Increased androgen levels can be reduced by the use of ketoconazole, dexamethasone, abiraterone ace-

tate or 5 α -reductase inhibitors. Antiandrogen withdrawal and enzalutamide can be used to counter the promiscuous AR escape route. The use of metformin, cetuximab or cabozantinib could represent ways to overcome the outlaw pathway, but further studies are needed to show the efficacy of these drugs in mCRPC. Bcl-2 inhibitors, emerging drugs still in experimental phase, show great potential in counteracting the bypass pathway. Docetaxel and cabazitaxel, the standard chemotherapy of mCRPC, are the treatment of choice when androgen-independent prostate cancer cells are selected (as supported by the lurker cell pathway). The correct and rational use of all these drugs may delay by months or even years the need to administer chemotherapy in patients with mCRPC but some AR targeted therapies may impair the subsequent response to chemotherapy.

Key words: androgen receptor, hormone refractory, prostate cancer

Introduction

Prostate cancer is the second most common diagnosed cancer in men worldwide, accounting for 14% of new malignancies and the sixth cause of cancer mortality, owing for 6% of cancer deaths in males [1,2]. In Europe, the corresponding figures in 2008 were 11.9 % and 9.3% [3].

Prostate cancer is an androgen-dependent disease as androgens stimulate the growth and survival and inhibit apoptosis of prostate cells, whether normal or tumorous. Androgen deprivation therapy (ADT) is based on the suppression of testis testosterone and it is the standard treatment

for patients with hormone-sensitive disease [4]. Other sources of androgens include the adrenals and the tumor cell itself. Castration-resistant prostate cancer (CRPC) is defined as progression of disease despite "castrate levels" of serum testosterone (<50 ng/dl or <1.7 nmol/l). The median time for progression from hormone-sensitive metastatic prostate cancer to CRPC is roughly 24 months [5]. Treatment of mCRPC is palliative but significant improvements have been made recently. Eventually, prostate cancer may become androgen-independent (anaplastic/ neuroendocrine disease), truly hormone-refractory, with median overall survival less than 1 year. However, this

is a rare event since most patients dying of prostate cancer still have PSA progression, meaning a functional AR, as PSA production is coded by a gene directly and exclusively regulated by the AR.

In this article we reviewed the subsequent hormonal manipulation (including novel drugs) in mCRPC, in relation to the AR-dependent growth mechanisms, evidence that supports the idea that the vast majority of mCRPC never become really hormone-refractory!

The androgen receptor

AR is a member of the nuclear receptor super-family. The AR protein consists of three functional domains: an amino-terminal activating domain, a central DNA-binding domain and a carboxy-terminal ligand-binding domain [6]. The AR can be activated by the testis, adrenals or tumor androgens. However, the wild-type AR is activated only by testosterone or dihydrotestosterone. AR mutations can broaden the ligand specificity to other steroids (estrogen, progesterone, corticosteroids) and even non-steroids (bicalutamide). The AR is bound in the cytoplasm to heat-shock proteins and other proteins in an inactive state. If an androgen binds to the hormone-binding domain of the AR, it initiates a cascade of events that activate the transcription of androgen-responsive genes and leads to the expression of different proteins crucial for prostate growth and cell survival. This cascade implies the dissociation of heat-shock proteins, hyper-phosphorylation, conformational changes, translocation into the nucleus, dimerization and association with co-modulator proteins [7,8]. Beside androgens, other processes can activate the AR, such as growth factors and regulatory signal pathways. The tumor environment and the immune system targeting strategies are beyond the scope of this paper.

Androgen deprivation therapy escape routes

Even if initially prostate cancer cells respond well to ADT, in the long run they become independent to circulating serum testosterone. Feldman and Feldman identified five mechanisms by which prostate cancer cells surpass ADT effects [9]. They are briefly described below and completed with a 6th one, as a basis for better understanding of second hormonal therapy options.

1. The hypersensitive AR

As response to low serum testosterone (TT) levels (due to ADT), three processes can occur:

a) AR amplification

Prostate cancer cells react by amplification of the AR gene and overexpression of the AR proteins. As a result, even low intraprostatic TT and dihydrotestosterone (DHT) levels could be sufficient for AR-dependent signalling [10].

b) Increased AR sensitivity

Prostate cancer cells increase the nuclear localization of the AR, increase the AR mRNA stability and upregulate the AR gene expression which results in greater sensitivity. Consequently AR is stimulated by four-times-lower levels of DHT than the LNCaP cell line, as shown by Gregory et al. [11].

c) Alterations of co-activators and/ or co-repressors

By amplification/ overexpression of the first and inhibition of the last ones [12].

2. The promiscuous AR

a) AR mutations

AR mutations generate a decrease in the specificity of ligand binding which translates into activation of the AR by non-androgen steroids, weak androgen precursors and even antiandrogens. These mutations can be observed in up to 20 % of CRPC [13].

b) Splice variants of AR

A series of splice variants of AR have been described, lacking the C-terminal ligand-binding domain but still able to activate known AR target genes and consequently contributing to prostate cell growth and survival [14,15].

3. The outlaw pathway

Outlaw receptors are steroid hormone receptors that are activated by ligand-independent mechanisms, such as the insulin-growth factor I, keratinocyte growth factor and epidermal growth factor, by tyrosine kinase cross-talk signalling via Ras-MAPKinase or PI3k-AKT-mTOR pathways [16].

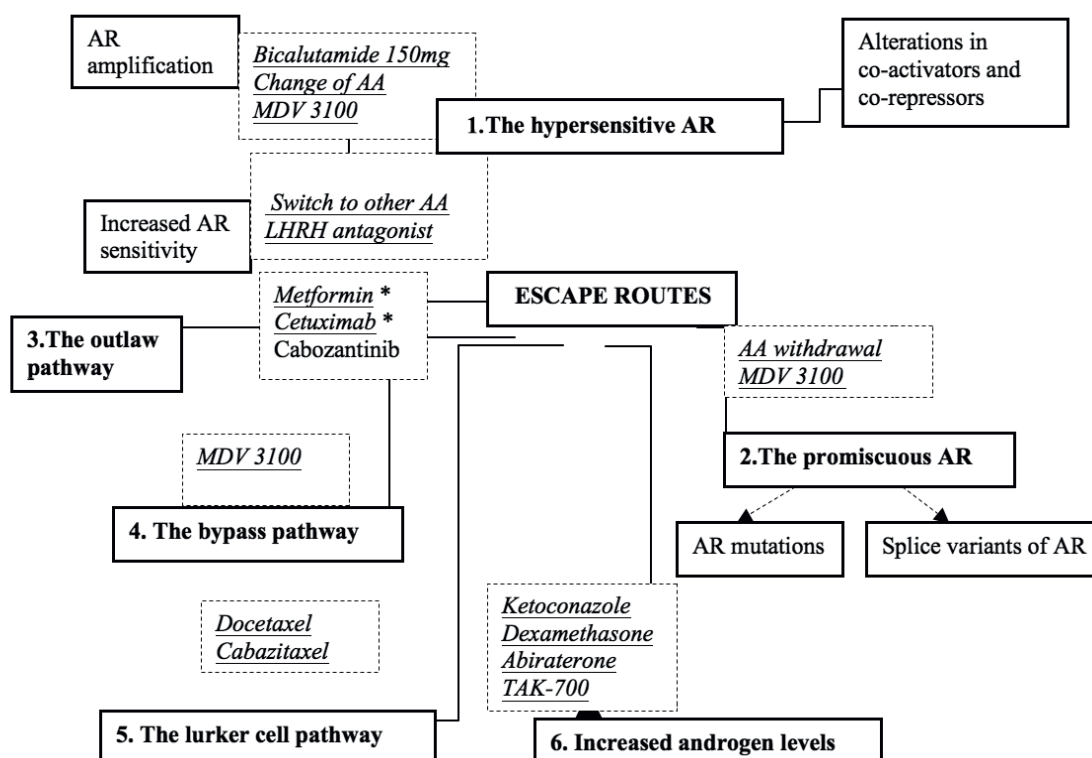


Figure 1. Hormonal escape mechanisms in prostate cancer and the therapy to circumvent them (* low evidence; AR: androgen receptor, MDV 3100: enzalutamide, AA: antiandrogens)

4. The bypass pathway

Is based on the existence of alternative pathways that are capable of bypassing the AR signal cascade when exposed to lack of testosterone, such as neuroendocrine differentiation, inactivation of PTEN suppressor gene or upregulation of Bcl-2 which protects cells from apoptosis [17,18].

5. The lurker cell pathway

Assumes the upfront existence of androgen-independent prostate cancer cells (probably progenitor CD44+/CD133+ tumor cells) and implies that those are selected and proliferate after initiation of ADT which kills only the hormone-sensitive ones [19].

6. We believe that *increased androgen levels* with “normal AR” should be individualized as an additional mechanism (intricate with 1b, but there the AR are hypersensitive), related to:

- increased 5 α -reductase activity which determines an increased conversion of testosterone into DHT;
- the peripheral conversion of adrenal steroids;
- intra-tumoral biosynthesis of androgens [20].

Implications for treatment

The interference of several new therapies with the escape routes of the classical hormonal sensitivity is depicted in Figure 1. Some drugs act on several escape routes.

1. a) AR amplification escape route can be fought by the use of a high-dose antiandrogen (bicalutamide 150 mg), change in antiandrogen preparation, the use of enzalutamide (MDV3100) or other AR antagonist (such as ARN-509, in phase I/II studies) and/or AR degraders (such as galaterone, still experimental).

Bicalutamide 150 mg (B150) given to patients with advanced prostate cancer who have progressed after conventional hormonal therapy (HT) induces a PSA response (decrease of a least 50%) and improves symptom status, including pain. SWOG 9235 was a phase II trial on (finally only 52) patients who had progressed after orchiectomy, LHRH analogue or diethylstilbestrol therapy, alone or in combination, and who never received any antiandrogen therapy or chemotherapy. The patients received B150 once daily. The authors reported a decrease in pain and an improvement in overall symptom status after 3 months of treat-

ment with B150, a PSA response in 20% of the patients and a median overall survival of 15 months [21]. There are only small retrospective reports but no trials available demonstrating that in patients with progression after conventional dose of bicalutamide (50mg/day) there is an increase in AR and that higher doses of bicalutamide should lead to a response in such patients. From a practical point of view, because of the risk that the PSA progression under “complete androgen blockade” might be due to the AR mutation (see also point 2 below), the antiandrogen withdrawal (AaWw) is a safer option and not to increase the bicalutamide dose. If no PSA decline is seen after 4 to 6 weeks of AaWw, for asymptomatic mCRPC patients with a good initial hormonal response (longer than 1 year, PSA nadir < 0.2 ng/ml) we can add again bicalutamide at higher dose (150 mg) to overcome the possible AR amplification, with close PSA check (every 1-2 weeks) and early stop of bicalutamide in the absence of PSA response.

Enzalutamide (MDV3100), an oral AR antagonist, prevents translocation of the AR to the nucleus, binding of the AR to DNA-responsive elements and the recruitment of co-activator proteins. Enzalutamide (ENZA) has no intrinsic agonist activity and induces tumor cell apoptosis. On 1199 patients with mCRPC progressing after chemotherapy and randomly assigned 2:1 to receive oral ENZA 160 mg per day or placebo, Scher et al. reported a longer median overall survival: 18.4 vs 13.6 months (HR= 0.63, $p < 0.0001$) [22]. They also showed a 54 vs 1.5% PSA response ($p < 0.001$), a higher soft-tissue response rate (29 vs 4%, $p < 0.001$), quality-of-life response rate (43 vs 18%, $p < 0.001$), a longer time to PSA progression (8.3 vs 3.0 months; HR 0.25; $p < 0.001$), a longer radiographic progression-free survival (PFS) (8.3 vs 2.9 months; HR 0.40; $p < 0.001$), and a longer time to the first skeletal-related event (16.7 vs 13.3 months; HR 0.69; $p < 0.001$) in the ENZA group vs the placebo group. This study (AFFIRM) led to the FDA approval of ENZA for mCRPC previously treated with docetaxel. The final results of the PREVAIL trial, comparing ENZA to placebo in chemonaive CRPC are pending.

b) Increased AR sensitivity can be countered if we further decrease the plasma androgens by:

- Switching to another AA;
- Further decrease of androgen levels by:
 - LHRH antagonists (abarelix, degarelix);
 - Change to another LHRH agonist;
 - Bilateral orchiectomy;

- Adrenals' inhibition (ketoconazole, steroids, abiraterone acetate, orteronel);
- Blocking testosterone synthesis (abiraterone acetate, orteronel).

LHRH antagonists bind immediately and competitively to LHRH receptors in the pituitary gland causing a rapid decrease in LH, FSH and consequently testosterone levels;

8-24 h after administration, LH level is reduced by 51-84%, FSH level by 17-42% and testosterone drops to castration levels in more than 80% of the patients. In contrast to LHRH agonists, the effect of LHRH antagonists installs in less than 24 h, they show no flare effect and after stopping them, and the recovery of the pituitary-gonad function is rapid [23]. Even if the use of LHRH antagonists seems appealing, it is encumbered by serious histamine-mediated side effects, lack of long term survival data and of depot formulations beyond 1 month. Trachtenberg et al. compared the endocrine and biochemical efficacy of abarelix (LHRH antagonist) with that of leuprolide (LHRH analogue) and a non-steroidal antiandrogen, and reported a rapid reduction of testosterone to castration levels on day 8 ($p < 0.001$) and a higher efficacy in avoidance of testosterone flare ($p < 0.001$) in favor of abarelix [24]. Phase III data comparing degarelix with leuprolide in prostate cancer metastatic to bone showed a lower risk of failure or death (composite endpoint) and more pronounced decrease in serum alkaline phosphatase in favor of degarelix [25].

Although we acknowledge that there is no evidence based data regarding the switch from LHRH agonists to LHRH antagonists or the change to another LHRH agonist or bilateral orchidectomy, we shall notice that any LHRH agonist has a 5-17% inability to induce castration levels of TT. We, like others [26], observed that by changing the LHRH agonist with another one or by performing a bilateral orchidectomy for mCRPC with non-castrate TT levels, we managed to drop the TT to significantly lower levels (below 20 ng/ml), with corresponding subsequent PSA response and clinical improvement.

The adrenals' inhibition and the enzymatic inhibition of testosterone synthesis will be discussed at point 6.

2. An example of promiscuous AR implication in clinical practice is represented by PSA decrease in approximately 30-40% of the patients who stopped taking the antiandrogen. Sartoro et al. re-

ported a >50% decrease in PSA levels in 21% of the patients who underwent antiandrogen withdrawal and a 12-month or greater progression-free interval in 19% of patients [27].

Enzalutamide, an oral AR antagonist, might also be useful in counteracting this escape route, as it exerts no agonistic properties *in vitro* in cell lines that have AR mutations that are stimulated by antiandrogens.

3. The use of metformin, cetuximab or cabozantinib could represent ways to overcome the outlaw pathway, but further studies should be performed in order to show the efficacy of these drugs in mCRPC.

Metformin, an oral biguanide used in the treatment of type II diabetes exerts anti-neoplastic and chemopreventive activities through the activation of AMP-activated protein kinase (AMPK), inhibition of the mammalian target of rapamycin (mTOR) pathway and inhibition of insulin like growth factors (IGFs) [28].

Cetuximab, an anti-EGFR monoclonal antibody, was shown to decline PSA levels and improve PFS in patients with mCRPC. In a phase II trial Cathomas et al. reported a 34% PFS at 12 weeks, a 13.3 months median OS and a >50% decline in PSA in 20% of their 38 patients [29].

Cabozantinib is a dual tyrosine kinase inhibitor, with activity against MET and vascular endothelial growth factor receptor 2, supposed to act synergistically in angiogenesis. Results of a phase II randomized trial showed regression in soft tissue lesions in 72% of the patients, improvement on bone scan in 68% of them and even complete resolution on bone scan in 12% of the patients. In this trial included were 171 men with mCRPC who received 100 mg of cabozantinib daily; patients with stable disease at 12 weeks were randomly assigned to placebo or going on cabozantinib. Of 171 patients, 31 with stable disease at week 12 went through randomization. In this group the authors reported a median PFS of 23.9 weeks (95% CI, 10.7-62.4) with cabozantinib vs 5.9 weeks (95% CI, 5.4-6.6) with placebo and improvement in bone pain in 67% of the patients (with a decrease in narcotic use in 56% on a retrospective review). The most common grade 3 adverse events were fatigue (16%), hypertension (12%), and hand-foot syndrome (8%) [30]. Phase 3 trials are launched (COMET 1 and COMET 2).

4. The bypass pathway is targeted by chemotherapy such as docetaxel (discussed later) or by

Bcl-2 inhibitors- emerging drugs in cancer therapy, still in experimental phase. Some intervene at the Bcl-2 checkpoints, others are humanized antibodies to death receptors, agents that target the inhibitors of apoptosis proteins, mimetic of small mitochondria-derived activator of caspases or are antisense therapies targeting cytoprotective chaperones. OGX-011 (custirsén), an inhibitor of the cytoprotective chaperone protein clusterin, is tested in the docetaxel-prednisone *Synergy* randomized phase 3 trial (completed) [31], as well as in an ongoing cabazitaxel phase 3 trial (*Affinity*).

5. The lurker cell pathway is intercepted by chemotherapy, currently indicated for symptomatic mCRPC or even asymptomatic if short PSA doubling time [32] and/ or visceral metastases are proven. For patients with good performance status, short response (less than 1 year) to first ADT or Gleason score 8-10 are factors for considering chemotherapy instead of other hormonal manipulations. Standard first line chemotherapy of mCRPC is docetaxel, second line is cabazitaxel [33-35], both inhibiting the AR activity [36]. Currently there is no standard third line, the alternatives being mitoxantrone or etoposide/ Cis(Carbo)-platin or, in selected cases, rechallenge with docetaxel. An overview of the chemotherapy trials is beyond the scope of this article. It should be noted that trials with docetaxel and cabazitaxel were conducted before new hormonal manipulations such as abiraterone or enzalutamide validated their efficacy. Therefore, from a principle point of view, probably chemotherapy should come in when all means of hormone therapy have been exhausted. However, recent preliminary and retrospective data showed that abiraterone decreases the efficacy of docetaxel [37] or cabazitaxel (presented yet only as posters, Angelergues A at ASCO 2013 and Sonpavde G at ECC 2013). *Vice-versa*, docetaxel but not cabazitaxel seems to diminish the efficacy of new AR targeted agents, but again only on low level of evidence (small number of patients and retrospectively, presented only as abstracts). Nevertheless, these results underline the complexity of drug-AR interactions and mandate identification of predictive biomarkers and run of randomized studies on optimal sequence therapies in mCRPC [38,39].

6. Increased androgen levels can be reduced by the use of ketoconazole, dexamethasone, abiraterone acetate or 5 α -reductase inhibitors. Ketoconazole, an oral antifungal agent, reduces adrenal

hormonal synthesis by inhibiting 11 β -hydroxylase, an enzyme that converts progesterone to 17 α -hydroxyprogesterone. The inhibition of adrenal cortical hormone synthesis is so effective that glucocorticoids replacement therapy is necessary. Even if ketoconazole is a strong inhibitor of both adrenal and testicular pathways of steroid synthesis, its use is shadowed by frequent side effects and drug-drug interactions. In a phase III randomized trial, Small et al. reported a better PSA response in patients who underwent antiandrogen withdrawal (AAWW) in combination with 1200 mg ketoconazole, but there was no difference in OS between the two study arms. In this trial, 260 patients were randomized between AAWW alone and AAWW+400 mg ketoconazole three times daily, with a PSA response in 11 vs 27% of the cases [40].

Similarly, low doses of dexamethasone (0.5-1 mg/ day, orally) induce adrenal inhibition. A more than 50% PSA decrease is noted in 25-49 % of patients, with PSA PFS of 8-11.6 months for responders [41,42]. It should be noted that steroids are the most common hormonal therapy in mCRPC, as prednisone was given aside with docetaxel, cabazitaxel and abiraterone in all major randomized trials. With the exception of abiraterone association (to reduce its mineralocorticoid effect), probably prednisone is not needed. In pre-clinical studies, glucocorticoids can activate AR mutation and increase expression of AR truncated splice variants.

Abiraterone acetate is an oral drug acting as a selective inhibitor of 17 α -hydroxylase-20 lyase (CYP17), an enzyme implicated in androgen biosynthesis in the prostate tumor tissues, adrenal glands and testis. Due to the CYP17 inhibition, TT synthesis is blocked and serum TT decline to castration levels. Abiraterone acetate could be a potent second-line therapy in patients with mCRPC after docetaxel failure and even replace docetaxel as first-line therapy, as it determines a minimum 50% decrease in PSA for 60% of chemotherapy-naïve mCRPC patients and shows consistent

antitumor activity in patients previously treated with chemotherapy [43]. COU-AA-301 is a randomized, double-blind, placebo-controlled phase III trial in which were included 1195 patients with mCRPC that progressed after docetaxel chemotherapy. Patients were randomly assigned (ratio 2:1) to receive either 1000 mg abiraterone once daily plus prednisone (5 mg orally twice daily) (797 patients) or placebo plus prednisone (398 patients). At a median follow-up of 20.2 months, the authors reported better outcomes for the abiraterone group (Table 1) [44]. The same doses of abiraterone and prednisone vs 10 mg prednisone as control group were tested in 1088 chemo-naïve mCRPC (COU-AA-302 trial). At 22.2 months median follow-up, Ryan et al. [45] concluded that abiraterone improved the radiographic PFS, showed a trend toward improved OS (because the pre-specified boundary for significance $p \leq 0.001$ was not reached at the observed number of events), and significantly delayed clinical decline and initiation of chemotherapy (25.2 vs. 16.8 months, $p < 0.0001$) in patients with mCRPC (Table 1). The main side effects of abiraterone are liver function abnormalities and mineralocorticoid-related hypokalaemia and fluid retention translating into possible severe hypertension and cardiac disorders. A challenger for abiraterone, without having the mineralocorticoid side effects, is orteronel (TAK-700) tested in the same scenario as abiraterone acetate in ongoing randomized trials. There is a primary resistance to abiraterone in 1 out of 4 patients [46], as well as for ENZA [47]. Due to the different mechanism of action, their association is tested, but is likely to be non cost-effective as abiraterone decreases the efficacy of ENZA and *vice-versa*, in retrospective data [48-50].

Conclusions

mCRPC shows a number of adaptive mechanisms that facilitate continued AR-dependent tumor growth, such as AR amplification, increased AR sensitivity, AR mutations, outlaw receptors parallel to the selection and proliferation of andro-

Table 1. Randomized trials comparing abiraterone acetate plus prednisone vs prednisone alone for mCRPC progressing during or after docetaxel (COU 301) or given before any chemotherapy (COU302)

Trial	Median overall survival (months)	Median time to PSA progression (months)	Median time to radiological progression (months)	PSA response (%)
COU 301 [44] (postchemo)	15.8 vs 11.2 (HR 0.74, $p < 0.0001$)	8.5 vs 6.6 (HR 0.63, $p < 0.0001$)	5.6 vs 3.6 (HR 0.66, $p < 0.0001$)	29.5 vs 5.5 ($p < 0.0001$)
COU 302 [45] (prechemo)	NR vs 27.2 (HR 0.75, $p = 0.01^*$)	11.1 vs 5.6 (HR 0.48, $p < 0.0001$)	16.5 vs 8.3 (HR 0.53, $p < 0.0001$)	62 vs 24 ($p < 0.001$)

HR: hazard ratio, PSA: prostate specific antigen, mCRPC: metastatic castration resistant prostate cancer *not reaching the pre-specified statistical significant value of 0.005 (i.e. not significant)

gen-independent prostate cancer cells. The variety of these mechanisms in mCRPC and the advent of new hormonal therapies allow us to individualize the treatment for each patient, redefining the hormone-refractory prostate cancer status and delaying chemotherapy for months or even years. The true hormone-refractory metastatic prostate cancer is a rare event, most mCRPC patients dying with PSA progression with an active AR! Therefore, maintaining castration levels of testosterone is mandatory

and should be checked regularly. We foresee that chemotherapy will have to challenge other new hormonal agents beside the already approved ones (abiraterone and enzalutamide). The optimal sequence remains to be defined.

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