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Mini-review

Androgen deprivation of prostate cancer: Leading to a therapeutic dead end

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ABSTRACT

Androgen deprivation therapy (ADT) is considered as the standard therapy for men with *de novo* or recurrent metastatic prostate cancer. ADT commonly leads to initial biochemical and clinical responses. However, several months after the beginning of treatment, tumors become castration-resistant and virtually all patients show disease progression. At this stage, tumors are no longer curable and cancer treatment options are only palliative.

In this review, we describe molecular alterations in tumor cells during ADT, which lead to deregulation of different signaling pathways and castration-resistance, and how they might interfere with the clinical outcome of different second-line therapeutics. A recent breakthrough finding that early chemotherapy is associated with a significant survival benefit in metastatic hormone-sensitive disease highlights the fact that there is time for a fundamental paradigm shift in the treatment of advanced prostate cancer. Therapeutic intervention seems to be indicated before a castration-resistant stage is reached to improve therapeutic outcome and to reduce undesirable side effects.

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Introduction

Prostate cancer is the most common cancer among men in industrialized countries and represents one of the third most leading causes of cancer deaths [1,2]. Whereas early stages of the disease can be successfully treated with surgery or radiation therapy, no curative treatment exists for advanced stages.

More than seventy years ago Huggins and Hodges recognized that prostate cancer (PCa) is an androgen-sensitive disease. Therefore, they established the bilateral orchiectomy as the earliest method to decrease the testosterone serum levels in an effort to slow down the tumor growth [3].

Today, androgen deprivation therapy (ADT) is considered as the standard therapy for men with *de novo* or recurrent metastatic disease [4]. It usually includes the chronic administration of gonadotropin-releasing hormone analogs, anti-androgens or their combination, also described as maximal androgen blockade. ADT commonly leads to an initial response with suppression of prostate specific antigen (PSA) levels in 80–90% of patients and objective responses in soft tissue and bone

metastases. It also prolongs overall survival and is able to alleviate bone pain [5].

Adverse effects of ADT include notable impairment of the quality of life, including sexual dysfunction, muscle atrophy, osteoporosis, hot flashes, fatigue, gynecomastia or anemia [6–8]. Moreover, in some patients ADT is associated with depression or cognitive dysfunction, pneumonia, acute kidney injury, increased incidence of diabetes, and cardiovascular morbidity and mortality [9–12]. Therefore, predisposing factors of patients, such as a history of diabetes or cardiovascular events, have to be regarded before ADT initiation. The fundamental problem, however, is that virtually all patients show disease progression at a median of 18–24 months after the beginning of treatment despite maintenance of castrate testosterone serum levels of less than 20 ng/dl [13]. This recurrent form of PCa has been termed castration-resistant prostate cancer (CRPC). The expected survival time after progression is only 16–18 months [14], and only 5–10% of patients remain alive 10 years after initiating ADT [15].

For a long time, treatment options that improved survival in this setting were limited to docetaxel-based regimens [16,17]. However, only about half of men with CRPC respond to docetaxel chemotherapy, and all of them eventually discontinue this treatment because of toxicity or disease progression [18]. Recent major advances have resulted in regulatory approval of enzalutamide (MDV1300) and abiraterone acetate (AA). Enzalutamide suppresses the androgenic action in prostate cancer cells by inhibiting the nuclear translocation, chromatin binding, and coregulator binding of the androgen receptor (AR) [19]. AA is a CYP17

Abbreviations: ADT, androgen deprivation therapy; AR, androgen receptor; CRPC, castration resistant prostate cancer; PCa, prostate cancer; PSA, prostate specific antigen.

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enzyme inhibitor blocking the androgen production in testes, adrenal glands, and tumor microenvironment [20]. Clinical studies showed that further AR suppression by these drugs can extend patient's survival. Unfortunately, overall survival after chemotherapy is only modest (4–5 months) and most responding patients relapse within 1–2 years with evidence of renewed AR activity [20–23].

The leading cause for the typical course of the disease under ADT is the formation of ADT resistant tumor cells. In this review, we describe molecular alterations of different signaling pathways during ADT and how they may influence the therapeutic action of drugs targeting these pathways.

Molecular alterations during ADT and their implication for therapeutic outcome

Initially, the continuous deprivation of androgens during ADT leads to a decrease in cell proliferation and growth as well as an increase of apoptosis, which finds expression in biochemical and clinical response.

However, in the course of time a variety of genetic alterations accumulate in some tumor cells and different signaling pathways are deregulated that influence the cellular outcome. The tumor cells

can now proliferate and grow at low androgen levels or completely androgen-independently and show a very aggressive behavior. At this stage, the anti-tumor activity of the ADT is neutralized, metastases progress and any curative treatment is no longer possible. In the past years, many of the molecular and genetic aberrations during ADT were identified and it became clear that multiple AR-dependent and -independent intracellular pathways are influenced on the way to castration resistance (CR) (Fig. 1). As a consequence, the alterations in cellular signaling during ADT can be associated with reduced therapeutic effects of drugs targeting these pathways.

AR signaling

Generally, androgens predominantly function through their binding to the AR, a member of the steroid hormone receptor family of ligand-activated nuclear transcription factors. In the inactive state the AR is bound to heat-shock proteins in the cytoplasm. After binding of testosterone or dihydrotestosterone (DHT), which binds in a more stable manner, the AR homodimerizes, undergoes phosphorylation and translocates to the nucleus. In the nucleus the AR can bind to androgen-responsive elements (ARE) in the promoter

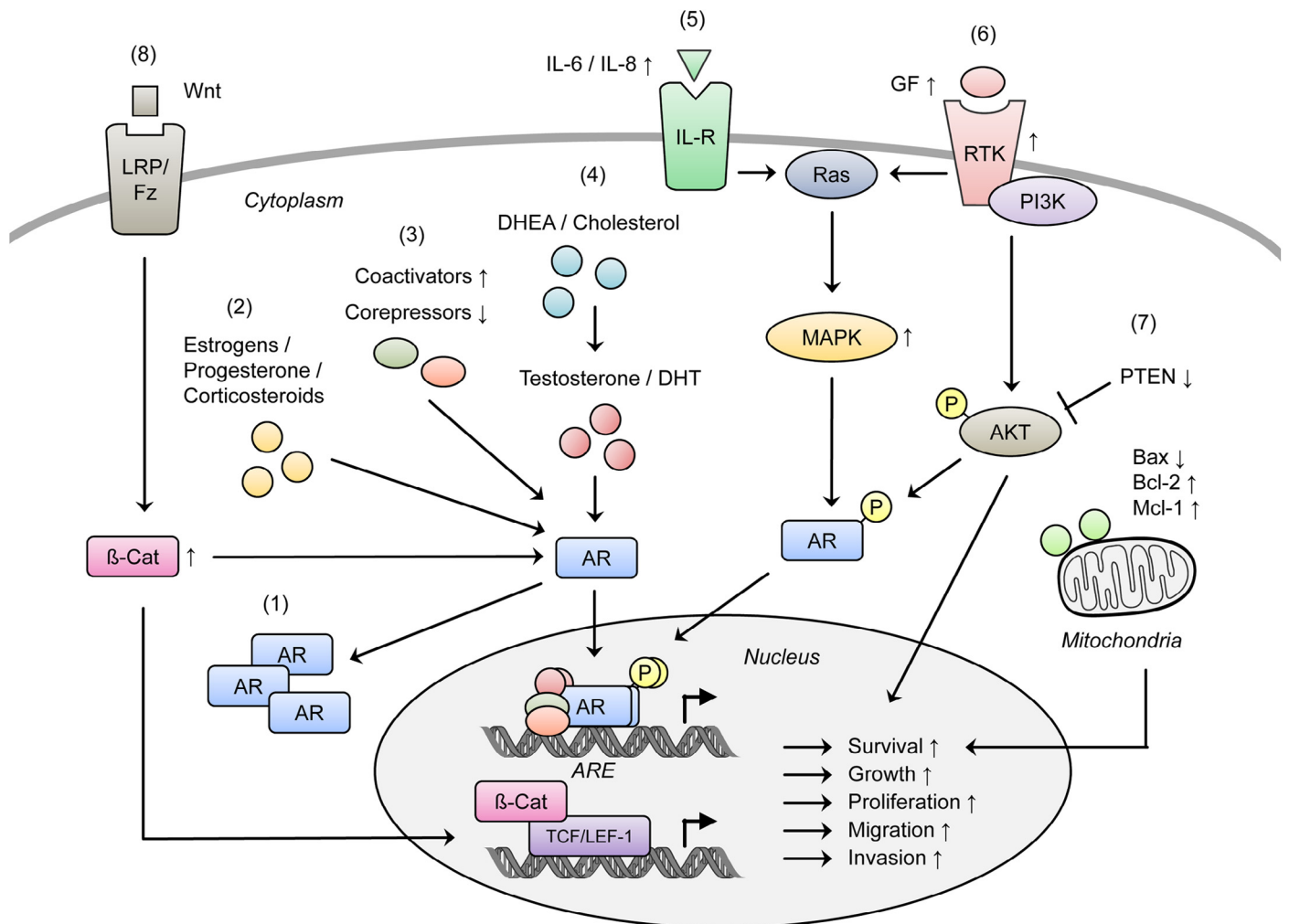


Fig. 1. Schematic representation of molecular alterations in castration resistant prostate cancer. (1) AR amplification, (2) binding of alternate AR ligands, (3) upregulation of AR coactivators and downregulation of AR corepressors, (4) intratumoral steroidogenesis, (5) enhanced activation of cytokine pathways, (6) enhanced growth factor signaling, (7) deregulation of pro- and anti-apoptotic proteins, (8) enhanced Wnt/ β -catenin signaling. Abbreviations: AKT, akt serine/threonine kinase; AR, androgen receptor; ARE, androgen responsive elements; β -Cat, beta-catenin; DHEA, dihydrotestosterone; DHT, dihydrotestosterone; GF, growth factor; IL-6, interleukin-6; IL-8, interleukin-8; LRP/Fz, low-density-lipoprotein-related protein/Frizzled receptor; MAPK, mitogen-activated protein kinase; P, phosphorylated residues; PI3K, phosphoinositide-3-kinase; PTEN, phosphatase and tensin homolog; Ras, rat sarcoma protein; RTK, receptor tyrosine kinase; TCF/LEF-1, t-cell specific transcription factor/lymphoid enhancer-binding factor; Wnt, wnt ligand; \uparrow , upregulation; \downarrow , downregulation.

regions of target genes. Then, in interaction with coactivator or corepressor proteins, the AR regulates the expression of the target genes involved in cell-cycle regulation and proliferation [24].

In a large portion of castration-resistant tumors, an enhanced expression of the AR was detected, which can result in a reactivation of AR transcriptional activity despite low androgen levels. In 20–30% of CR prostate tumors the enhanced AR expression is based on AR gene amplifications [25,26]. Moreover, gain of function mutations in the AR gene, mostly located in the ligand binding domain, lead to mutants, which have an increased affinity to androgens or which can be activated by alternative steroidal molecules, like estrogens, progesterone or corticosteroids [27–29].

The transcriptional activity of the AR can also be altered by a deregulated expression of coactivator or corepressor proteins, which enhance or reduce AR transactivation, respectively. For example, the coactivators Tif2, SCR1 and Tip60 are overexpressed in CRPC samples [30,31]. In recent studies, a decreased expression of the corepressors SMRT/NCoR and EBP1 was demonstrated [32,33].

An enhanced activation of AR cell signaling pathways is also possible through an intratumoral testosterone synthesis during ADT. This may be caused by an enhanced gene expression of numerous enzymes mediating intracellular androgen metabolism from adrenal dehydroepiandrosterone (DHEA) or androstenedione to testosterone and dihydrotestosterone (DHT) [34,35]. Furthermore, proteins involved in cholesterol homeostasis are altered in androgen-independent growing tumor cells in a manner that appears to be generating free cholesterol that may be used to provide precursor to the steroidogenic pathway [36].

Ligand-independent activation of the AR is usually based on post-translational mechanisms, especially phosphorylation, which relate to persistent AR activity. Guo et al. suggested that the Src-induced phosphorylation of the AR at Y534 could lead to an androgen-independent activation of the AR [37]. Kraus and colleagues demonstrated that the adaptor/scaffolding protein receptor for activated C kinase 1 (RACK1) in interaction with Src modulates the transcriptional activity of the AR by phosphorylation [38]. Moreover, tyrosine kinase Etk, a downstream effector of Src, is upregulated during androgen depletion, which is followed by an increased stability and enhanced activity of the AR [39].

The nuclear factor NF- κ B signaling pathway regulates the expression of interleukin-6 (IL-6) and interleukin-8 (IL-8). Both cytokines are also known to stimulate AR activity. The NF- κ B pathway was shown to be upregulated in many CRPC, and in *in vitro* experiments, an increased NF- κ B signaling resulted in an enhanced AR activation [40].

Taken together, the activation of AR-dependent pathways in PCa cells during ADT hinders the effective further use of anti-androgens and contributes to restore AR activity after enzalutamide or AA treatment [41]. Interestingly, evidence has suggested that taxanes also exert their antineoplastic activity in prostate cancer partly by blocking AR signaling [42], so that overexpression, amplification and mutation of the AR may also play a role in the development of a chemoresistant state [43].

Growth factor signaling

Growth factor receptors have receptor tyrosine kinase (RTK) activity. After ligand binding, they are able to activate signaling pathways resulting in an activation of transcription factors and an altered expression of various genes responsible for cell growth, proliferation and survival. Increases in autocrine and paracrine growth factor loops were described during ADT at low or even near-zero concentrations of androgens. As a consequence, essential growth and survival pathways are deregulated. For example, the epidermal growth factor (EGF), the transforming growth factor α (TGF α), the insulin-like growth factor-1 (IGF-1), the keratinocyte growth factor (KGF) or the basic

fibroblast growth factor (bFGF) as well as their corresponding receptors were shown to be overexpressed in CRPC [44–48]. Moreover, increased expression and activity of the mitogen activated protein kinase (MAPK), a key mediator of growth factor signaling, was shown in recurrent tumors of castrated mice [49].

Overexpression of the EGF receptor (EGFR, ErbB-1) is correlated with the time of biochemical relapse and immunohistochemical examinations showed a statistical correlation between EGFR expression and higher serum PSA [50,51]. Interestingly, there seems to be a paracrine activation of EGFR on androgen-dependent tumor cells by TGF α from the surrounding stromal cells. In contrast, in CRPC metastases, the tumor cells express EGFR as well as TGF α , leading to an autocrine regulation of the intratumoral signaling pathways [44].

The RTK Her-2/neu (ErbB-2) was also shown to be overexpressed in androgen-independently growing cell lines as well as in sublines that have been xenografted into castrated mice [52,53]. Her-2/neu overexpression can lead to an activation of AR related genes through the Akt pathway in the absence of androgens [54], which results in an enhanced metastatic and angiogenic potential of the tumor cells [55,56]. It is suggested that the overexpression of RTKs of the ErbB family and the autocrine activation of their related signaling pathways might be the reason why studies with ErbB inhibitors (e.g. erlotinib, gefitinib) or anti-ErbB antibodies (e.g. trastuzumab, pertuzumab) alone or in combination with docetaxel have not been encouraging in patients with CRPC [57–62].

Binding of the hepatocyte growth factor (HGF) from prostate stroma to the HGF-receptor (HGFR, c-met) of prostate cancer cells promotes proliferation, motility and invasion and is associated with bone metastasis [63]. Normally, the expression of c-met is downregulated by the AR [64]. However, during long-time ADT an overexpression of c-met was observed, which is associated with a more aggressive disease [65]. Cabozantinib, an inhibitor of c-met and VEGFR2, was shown to improve metastatic lesions and to increase progression free survival of patients with CRPC [66]. However, preliminary results from a pivotal phase III study have failed to produce survival benefit [67].

Apoptotic signaling

Apoptotic pathways have a profound effect on the malignant phenotype and oncogenic mutations disrupt apoptosis, leading to tumor initiation, progression and metastasis. Dysregulation of apoptotic signaling pathways is also associated with progression to CRPC, reflecting the consequence of blocking the programmed cell death that would normally ensue upon ADT.

The expression of several anti-apoptotic members of the bcl-2 gene family, including bcl-2, bcl-X, and mcl-1, is enhanced during progression of prostate tumors, a finding that seems to be relevant to the hormone-insensitive, metastatic phenotype [68]. In immunohistological studies, a high Bcl-2 expression was associated with higher Gleason scores and lower biochemical-free survival in patients with advanced PCa undergoing ADT [69]. There is evidence that Bcl-2 expression and its pro-survival response are regulated by the TNF- α /NF- κ B signaling pathway. The addition of TNF- α to LNCaP prostate carcinoma cells caused a 40-fold increase in bcl-2 promoter activity and an augmentation of increased Bcl-2 levels after hormone withdrawal. This effect was abated by the addition of NF- κ B inhibitors [70].

In a recent study, the pathological role of Myb, a transcription factor, which is known to be overexpressed in CRPC, was examined. Myb overrode androgen-deprivation induced cell cycle arrest and apoptosis in prostate cancer cells by induction of Bcl-2 and Bcl-xL as well as downregulation of p27 and the proapoptotic protein Bax. As a result, prostate cancer cells developed an enhanced motility and invasion as well as an increased potential in epithelial-mesenchymal transition (EMT), suggesting a functional role of Myb

overexpression in the malignant and aggressive behavior of tumor cells during ADT [71].

Bcl-2 is also known to contribute to docetaxel resistance. For this reason, a phase II study investigated the activity of oblimersen sodium, a bcl-2 antisense oligonucleotide, administered before docetaxel to patients with CRPC. PSA response was observed in 46% and 37% of patients treated with docetaxel and docetaxel-oblimersen and partial response according to RECIST criteria was achieved in 18% and 24% of patients, respectively. However, the primary end points of the study (confirmed PSA response >30%; major toxic event rate <45%) could not be met [72].

The proapoptotic protein PTEN (Phosphatase and Tensin homolog) is also frequently mutated or inactivated in CRPC [73]. PTEN is classified as a regulator of the phosphoinositide 3-kinase (PI3K)/Akt/mTOR cell-survival pathway, which is one of the most critical in human cancer [74]. Various growth factors, like IGF and FGF, can induce this pathway, leading to the activation of PI3K and formation of PIP3. PIP3 phosphorylates AKT. Phosphorylated pAKT in turn activates multiple molecules involved in cell proliferation and survival. PTEN catalyzes the dephosphorylation of PIP3 to PIP2, leading to an inhibition of the pathway [75]. The relevance of PTEN loss to the development of castration-resistant cell clones was emphasized by experiments with transgenic mice characterized by prostate-specific inactivation of PTEN. They all developed adenocarcinoma and some of them lymph node or lung metastases within 29 weeks. Following castration, an immediate initial increase of apoptosis was seen, that was gradually replaced over time by an outgrowth of PTEN null castration-resistant proliferative cell clones [76].

Makhov and colleagues showed that a decreased PTEN expression led to sunitinib resistance in prostate cancer cells [77]. Sunitinib is a tyrosine kinase inhibitor, which exhibits impressive activity against advanced renal cell carcinoma. However, a phase III study with sunitinib in combination with prednisone in patients with CRPC after docetaxel-based chemotherapy was discontinued due to lack of OS prolongation [78].

Wnt/ β -catenin signaling

The canonical Wnt/ β -catenin pathway, which is originally involved in the preliminary development of the prostate, seems also to be implicated in the development of CRPC [79]. In a study of de la Taille, abnormal expression of β -catenin, the downstream effector of this pathway, was observed in 23% of tumor samples after radical prostatectomy and in 38% of CRPC samples [80]. β -Catenin leads to an upregulation of different genes, involved in tumor dissemination, metastasis and angiogenesis. Moreover, it is a central component of cadherin cell adhesion complexes, which have a critical role in the development of EMT [81].

Interestingly, a crosstalk between β -catenin and AR signaling was found. Generally, the androgen activated AR and the T-cell-specific transcription factor/lymphoid enhancer-binding factor 1 (TCF/LEF-1) compete for binding to β -catenin. In the presence of androgens, a formation of AR/ β -catenin complexes is driven, leading to the expression of AR related target genes. During ADT, when androgen-bound AR is diminished or absent in the nucleus, a complex formation between the transcription factor TCF/LEF-1 and β -catenin is preferred. This complex leads to an enhanced expression of TCF/LEF-1 target genes such as AR, c-myc, matrix metalloproteinase 7 (MMP7) or vascular endothelial growth factor (VEGF), which promotes tumor progression and metastasis [81]. Based on these facts, it cannot be ruled out that the enhanced VEGF expression induced by the Wnt/ β -catenin pathway is one reason why the addition of bevacizumab, a monoclonal antibody blocking VEGF, to docetaxel plus prednisone failed to improve the median OS of patients with CRPC [82].

Enrichment of PCa stem cell/progenitor cells

In recent years, the characteristics of PCa stem and progenitor cells under ADT were investigated. Interestingly, in castrated TRAMP mice, overexpression of Bcl-2 and the stem cell-like markers Sca-1, CD133 and KIT was observed at an early stage of 10 weeks after castration, while the expression was decreased and returned to the basal levels after 20 weeks. This was accompanied by a temporary cessation of tumor growth followed by a rapid tumor regrowth and increased metastasis until the end of the experiment after 36 weeks [83,84]. It is therefore suggested that immature PCa cells could undergo a preferred selection under ADT. Further studies are needed to evaluate their role in the recurrence of tumors after treatment failure.

Conclusions and future directions

Despite innumerable studies in the past decades, in which various modalities of ADT were tested in patients with advanced PCa, no one was cured from the disease with this treatment option until today. Moreover, the present second line treatments only bring slight success in view of an enhanced quality of life (QoL) and only minimal improvement in view of OS [16,17,20–23].

As demonstrated in the previous sections, molecular events for altered signaling, gene expression and cellular outcome with regard to survival, growth, proliferation, migration and invasion occur as a response to the androgen deprivation. This promotes the selection of tumor cells, which show an aggressive behavior and an enhanced propensity to metastasize [83,85,86]. In a clinical trial with AA, 79% of PCa patients with CRPC showed a decline in PSA serum level of 50% or more after 12 weeks of treatment. However, 52% of PCa patients simultaneously showed increased signs of bone lesions [87]. Other studies demonstrated an increased expression of aggressive markers in PCa patients with CRPC, such as N-cadherin [88], cadherin-11 [89], and nestin [90]. Interestingly, the increase of metastasis in the AA study was observed at a stage when the PSA levels of patients were significantly lowered [87]. This might contradict the general concept that the AR-regulated PSA rise during ADT is the early sign before PCa progresses to enhanced metastasis. Instead of this, this confirms that AR-independent pathways are also altered during ADT that might promote metastasis.

Causal connections can be established between molecular changes in cellular pathways during ADT and the therapeutic failure of drugs targeting these pathways [43,57,67,77]. Clinical testing of new drugs in patients with CRPC can therefore not provide sufficient evidence, whether these drugs could be more effective before or during ADT. Moreover, no statement can be given whether such drugs could be applied in a lower dosage before or in combination with ADT with less adverse effects or even whether they could result in cure.

Recent studies show evidence that the treatment of patients with advanced PCa can be markedly improved by therapeutic intervention before the castration resistant stage is reached. Ganitumab, an antibody targeting the IGF receptor, showed enhanced antitumor activity in combination with ADT in a mouse PCA xenograft model [91]. In a preclinical study of Thomas and colleagues, the combination of the AKT inhibitor AZD5363 with the antiandrogen bicalutamide enabled disease regression that endured longer than either monotherapy [92].

Impressive results were reached in the recent phase III CHAARTED (Chemohormonal Therapy versus Androgen Ablation Randomized Trial for Extended Disease) study. Patients with untreated hormone-sensitive PCa received ADT plus docetaxel (75 mg/m² 6 cycles every 3 weeks) or ADT alone (control group) [93]. The combination therapy significantly improved the OS from 44.0 to 57.6 months (HR 0.61, 95% CI 0.47–0.80; p = 0.0003). In subgroup analyses, patients with high volume metastatic disease (65%), as defined by the presence of visceral metastasis or ≥ 4 bone metastases (with at least one being extra-axial), had an even more dramatic OS improvement of 17 months with ADT plus

docetaxel (49.2 months vs. 32.2 months, HR 0.60, 95% CI 0.45–0.81; $p=0.0006$). This is in strong contrast to the usage of docetaxel as second line therapeutic in patients with CRPC, where only a mean OS improvement of 2–3 months could be achieved [16,17].

Effective treatment of advanced prostate cancer remains very challenging and much effort has to be undertaken to further understand the mechanisms of resistance. In the future, the situation for patients with advanced PCa could be improved further in the context of a personalized treatment [18,94]. A growing number of biomarkers, detected in tumor cells, blood or urine, could give information about the molecular phenotype of a tumor and could provide the basis for the decision, which agent(s) will be most effective for a given patient [94–96]. Moreover, they would help to find the right time to initiate the treatment and would support subsequent clinical monitoring.

Taken together, it seems to be time for a fundamental paradigm shift in the treatment of advanced PCa. Therapeutic intervention seems to be indicated for higher efficacy before a castration-resistant stage is reached. A statement of C. J. Sweeney, lead investigator of the CHAARTED study, boils it down to an essence: “Treating castration-resistant disease is a little bit defeatist (because it is already become resistant), let us try and start treatment early when it is more at risk of being cured – let us go forward by moving backward” [97].

Conflict of interest

None.

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