Metastatic Prostate cancer: current and emerging therapies

Kevin Courtney, MD, PhD
Assistant Professor of Internal Medicine
Division of Hematology/Oncology

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Kevin Courtney, MD, PhD
Assistant Professor of Internal Medicine, Division of Hematology/Oncology

Dr. Courtney is a member of the Simmons Cancer Center and co-leader of the Genitourinary Oncology Disease Oriented Team. His research is centered on supporting a robust translational oncology effort in genitourinary cancers, particularly prostate and kidney cancer, at UT Southwestern. In addition to his research into the metabolism of prostate and kidney cancer, Dr. Courtney is the principal investigator or lead site investigator on investigator-initiated, cooperative group-sponsored, and industry-sponsored clinical trials in genitourinary malignancies.

Purpose and Overview: This presentation reviews the biologic drivers of prostate cancer and resistance to castration therapy and examines recent advances and emerging potential therapies for the treatment of patients with metastatic castration-resistant prostate cancer (mCRPC).

Educational Objectives:

- To understand the mechanisms underlying the development and progression of prostatic adenocarcinoma
- To become familiar with current treatment options for metastatic prostate cancer and their biologic rationale
- To understand how our growing knowledge of the molecular mechanisms of resistance to current therapies is driving emerging clinical research

Introduction

Prostate cancer is the most frequently diagnosed malignancy and second leading cause of cancer death among men in the United States, with an estimated 233,000 new cases and 29,480 deaths in 2014 [1]. An estimated 1 in 7 men will develop prostate cancer [1]. Androgen deprivation therapy (ADT) through surgical or chemical castration has been the standard of care for the initial treatment of patients with metastatic prostatic adenocarcinoma since the seminal work of Huggins and colleagues reported more than 70 years ago [2,3]. However, the majority of patients with metastatic prostate cancer experience disease progression despite castration. Castration-resistant prostate cancer (CRPC) is principally responsible for prostate cancer mortality, and fewer than 20% of patients with CRPC survive beyond three years [4].

Androgen signaling continues to play a critical role in prostate cancer progression following castration. Recent efforts to better understand the roles that extra-gonadal androgen synthesis and the androgen receptor (AR) play in CRPC have led to the approval of promising new agents for CRPC. Additional therapeutic targets have emerged, including microtubules, growth factors, immune regulatory cells, and osteoblasts / osteoclasts, many of which exhibit a complex interplay with androgen signaling. Five therapies have received FDA approval based on improvement in overall survival for patients with metastatic CRPC in just the last 4 years (Table 1). Here we review the biologic drivers of prostate cancer and resistance to castration therapy. We will examine recent advances and emerging potential therapies for the treatment of patients with metastatic castration-resistant prostate cancer (mCRPC).

Agent	Action	Year	Primary Endpoint
Estramustine	Nitrogen mustard-estradiol conjugate	1981	Disease responses
Strontium-89	Radiopharmaceutical	1993	Pain palliation
Mitoxantrone + prednisone	Type II topoisomerase inhibitor	1996	Pain palliation
Samarium-153	Radiopharmceutical	1997	Pain palliation
Zoledronic acid	Bisphosphonate	2002	Reduced SRE
Docetaxel + prednisone	Microtubule stabilizer	2004	Overall Survival
Sipuleucel-T	Cell-based immunotherapy	2010	Overall Survival
Cabazitaxel + prednisone	Microtubule stabilizer	2010	Overall Survival
Denosumab	mAb to RANKL	2010	Reduced SRE
Abiraterone + prednisone	CYP17 inhibitor	2011, 2012*	Overall Survival
Enzalutamide	AR antagonist	2012, 2014*	Overall Survival
Radium-223	Radiopharmaceutical	2013	Overall Survival

Table 1. Currently approved therapies for mCRPC. SRE, skeletal-related event; mAb, monoclonal antibody; RANKL, receptor of nuclear factor kappa-B ligand; * dates are for FDA approval for treatment of patients post- and pre-docetaxel, respectively. Modified and updated from Modified from Pal SK, Lewis B, and Sartor O. Urol Clin N Am 2012; 39:583-591.

Androgen signaling and prostate cancer

The hypothalamus synthesizes and releases luteinizing-hormone-releasing hormone (LHRH), also known as gonadotropinreleasing hormone (GnRH), which stimulates the pituitary to release luteinizing hormone (LH) and adrenocorticotropic hormone (ACTH) (Figure 1)[5]. ACTH induces adrenal gland production of androgens from steroid precursors, while LH stimulates testosterone production by Leydig cells in the testis. In the prostate, 5α-reductases convert testosterone to the more potent androgen dihydrotestosterone (DHT) [6-8]. Androgen engagement of the androgen receptor (AR), a member of the nuclear receptor superfamily that serves as a ligand-dependent transcription factor, results in dimerization and nuclear translocation[9]. Androgen-engaged AR interacts with coregulatory proteins and binds androgen response elements (AREs) on DNA, resulting in transcriptional activation and repression of a host of genes that contribute to the development and progression of prostate cancer.

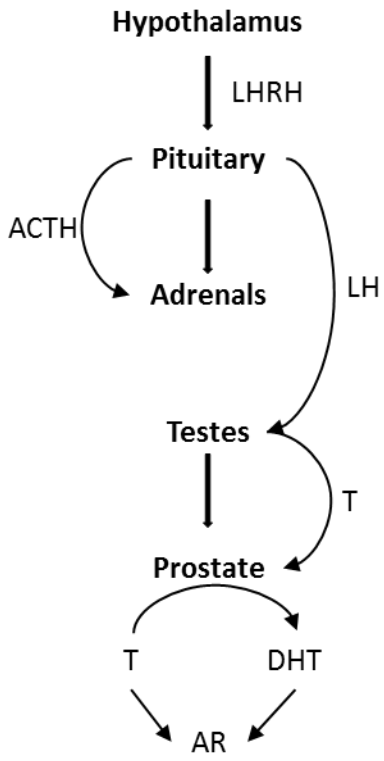

Figure 1. Endocrine axis in prostate cancer. LHRH, luteinizing hormone releasing hormone; LH, luteinizing hormone; ACTH, adrenocorticotropic hormone; T, testosterone; DHT, dihydrotestosterone; AR, androgen receptor.

ADT (castration) has long been the standard therapy for initial treatment of metastatic prostate cancer. In 1941, Dr. Charles Huggins and his colleagues reported on the beneficial effects of surgical castration or administration of estrogen to patients with metastatic prostate cancer[2,3]. Dr. Huggins shared the Nobel Prize in Physiology or Medicine in 1966 for his work on hormonal treatments of prostate cancer. Building on this work, the Veterans Administration Cooperative Urologic Research Group (VACURG) compared the synthetic oral estrogen diethylstilbestrol (DES) with orchiectomy and determined DES to be as effective as orchiectomy in the treatment of prostate cancer[10,11]. However, DES was associated with cardiovascular and thromboembolic toxicity, necessitating improved systemic therapy for metastatic prostate cancer[11]. Dr. Andrew Schally first characterized the structure of LHRH and generated synthetic peptide agonists to facilitate castration, for which work he was awarded the Nobel Prize in Physiology or Medicine in 1977[11,12].

Mechanisms underlying the development of CRPC

Castration is achieved through orchiectomy, or with LHRH agonists or antagonists. Castration halts the testicular production of testosterone. However, low levels of extra-gonadal androgens persist despite ADT, resulting in sufficient levels of testosterone and DHT to activate the androgen receptor in CRPC [13]. Additionally, elevated levels of testosterone have been measured in metastases from CRPC as compared to levels in primary tumors from non-castrate patients

[14]. Sites of extra-gonadal androgen synthesis include the adrenal glands and prostate cancer cells [15]. Prostate cancer cells may develop resistance to castration through several mechanisms. These include alterations in androgen uptake, increased expression of key enzymes involved in androgen synthesis, AR amplification or increased AR expression, the occurrence of AR splice variants, and mutations that increase AR activity [16-24]. Consequently, there are multiple sites for potential therapeutic intervention in the androgen signaling cascade. Further, we can take advantage of mechanisms that underlie interactions between androgen signaling and microtubules required for cell division that provides biologic incentive for targeting microtubules, the expression of prostate cancer-specific antigens that can afford targets for immunotherapy, and the interplay between prostate cancer cells and the bone microenvironment that opens the door for bone-targeted therapies for metastatic CRPC.

Impairing extra-gonadal androgen synthesis

More than 70 years ago, Charles Huggins and his colleagues recognized the potential role played by adrenal androgens in what we now term CRPC [2]. Huggins and colleagues demonstrated that bilateral adrenalectomy could lead to objective clinical responses in patients with metastatic CRPC [25]. The steps involved in androgen synthesis by the adrenal glands, and by prostate cancer cells themselves, subsequently have been more clearly defined. Figure 2 depicts the steps involved in androgen synthesis and key opportunities for therapeutic intervention [26].

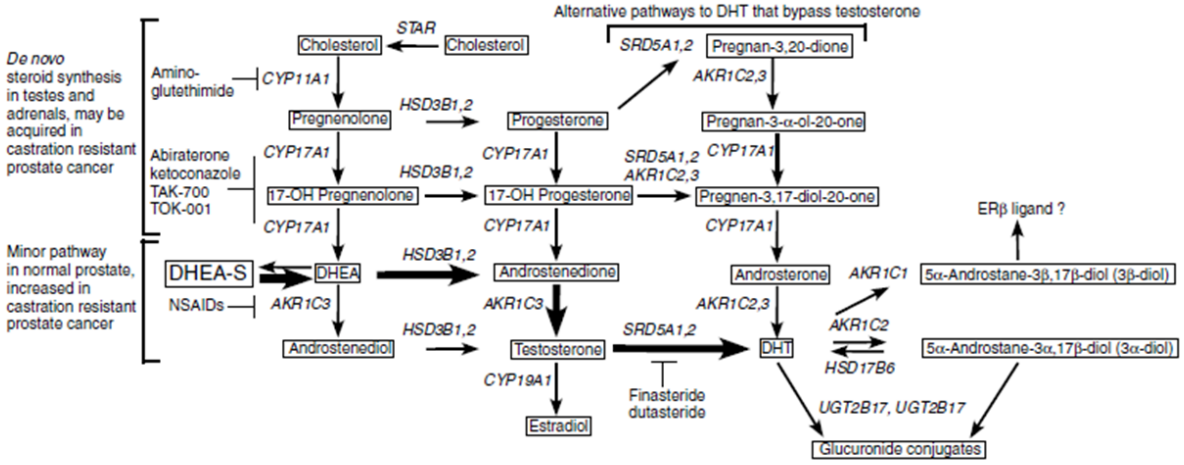

Figure 2. Androgen synthesis and metabolism in prostate cancer cells. Inhibitors of enzymes in the androgen synthesis pathways are indicated. From Cai C and Balk SP. Endocr Relat Cancer 2011; 18:R175-R182.

There has been longstanding interest in aminoglutethimide and ketoconazole for their anti-androgenic effects. Aminoglutethimide inhibits production of pregnenolone from cholesterol by CYP11A1, as well as the conversion of androgens to estrogens by aromatase [26,27]. Ketoconazole is a weak competitive inhibitor of several cytochrome P450 enzymes involved in androgen synthesis, including CYP17 (17α-hydroxylase / 17,20-lyase) and CYP11 [19,26,28] (Figure 2). Both agents have demonstrated biochemical and clinical activity in CRPC, supporting the hypothesis that adequate inhibition of enzymes upstream of testosterone and DHT synthesis could lead to improved outcomes in CRPC [27,29]. Abiraterone acetate, TAK-700, and TOK-001 are novel inhibitors of androgen biosynthesis.

Abiraterone acetate

Abiraterone acetate is a potent, selective, irreversible inhibitor of CYP17 (17α-hydroxylase / 17,20-lyase) [30] which has recently been approved for CRPC. A phase I study of abiraterone acetate in patients with CRPC provided confirmatory clinical evidence that CRPC can remain dependent on ligand-activation of the androgen receptor [30]. The phase I results demonstrated a plateau in the apparent pharmacodynamic effect was observed at the 1000 mg dose, which was chosen for subsequent studies [30]. Activity was observed at all dose levels, and abiraterone acetate was well-tolerated, with the predominant toxicities being those associated with mineralocoid excess: hypertension, hypokalemia, and edema [30]. This is consistent with the anticipated on-target mechanism of action of abiraterone acetate, as predicted by the observation that patients with congenital CYP17 deficiency exhibit loss of negative feedback control of adrenocorticotropic hormone (ACTH) [30].

To minimize the effects of impaired corticosteroid production and loss of negative feedback on ACTH, steroids were co-administered with abiraterone acetate in a phase II study of patients with docetaxel-refractory CRPC performed by Danila and colleagues [31]. Patients received abiraterone acetate 1000 mg daily and prednisone 5 mg twice daily to suppress symptoms of secondary hyperaldosteronism. Notably, a reduced incidence of hypokalemia and hypertension was appreciated with the addition of prednisone, which was adopted for the phase III study.

Abiraterone acetate received FDA approval in 2011 for the treatment of patients with docetaxel-refractory, metastatic CRPC based on an improvement in overall survival (OS) associated with abiraterone acetate in a randomized, phase III study [32]. Patients received abiraterone acetate 1000 mg daily plus prednisone 5 mg BID or placebo plus prednisone in a 2:1 ratio (779 vs 398 study participants). The primary endpoint was OS. Secondary endpoints included PSA response rate, time to PSA progression, and progression free survival (PFS). With a median follow up of 12.8 months, abiraterone acetate resulted in improved OS of 14.8 months vs. 10.9 months for placebo (HR 0.65, P< 0.001) [32]. The PSA response rate (29% vs. 6%, P<0.001), time to PSA progression (10.2 months vs. 6.6 months, P<0.001), and PFS (5.6 months vs. 3.6 months, P<0.001) all favored the abiraterone acetate [32]**. Mineralocorticoid-related adverse events of fluid retention, hypertension, and hypokalemia were more frequent in the abiraterone acetate arm [32]. FDA approval for abiraterone acetate plus prednisone was expanded in 2012 to include the treatment of men with chemotherapy-naïve metastatic CRPC. This was based on results of a 1:1 randomized, double-blind, phase III trial of 1088 men with metastatic CRPC with co-primary endpoints of radiographic PFS and OS showing and improvement in median PFS of 8.2 months (16.5 vs 8.3 months, HR 0.53, P<0.001) and an improvement in OS of 5.2 months at the third interim analysis (35.3 vs 30.1 mos, HR 0.79)[33].

TAK-700 (Orteronel)

TAK-700 is a reversible CYP17 inhibitor with preferential inhibition of 17,20-lyase over 17-hydroxylase activity [34,35]. Selective inhibition of 17,20-lyase activity may in theory reduce the need for corticosteroid supplementation, as secondary mineralocorticoid excess induced by CYP17 inhibition may be more dependent on 17-hydroxylase [35]. Despite promising results from

early phase studies, two randomized, double-blind phase III trials of TAK-700 plus prednisone vs. placebo in patients with either chemotherapy-naïve or docetaxel-refractory metastatic CRPC (clinicaltrials.gov ID: NCT01193244 and NCT01193257, respectively) failed to demonstrate a statistically significant improvement in overall survival [36,37].

Antagonizing androgen receptor function

The anti-androgens bicalutamide, nilutamide, flutamide are frequently used in primary ADT or added to castration therapy for CRPC. However, in the setting of AR overexpression or mutation, these "first generation" anti-androgens can serve as agonists and promote prostate cancer progression [16]. Indeed after prolonged exposure, some patients can experience a therapeutic anti-androgen withdrawal effect [29]. Additionally, AR splice variants have been identified that are constitutively active and may be able to increase the expression and activity of full-length AR to confer castration resistance [21]. These findings emphasize the need for agents that more effectively target AR changes that evolve with the development of resistance.

Enzalutamide

Enzalutamide is an AR signaling inhibitor with increased AR affinity compared to bicalutamide that functions as a pure antagonist [38]. Enzalutamide impairs AR nuclear translocation, DNA binding, and co-activator recruitment [38]. Enzalutamide received FDA approval in 2012 for the treatment of men with docetaxel-refractory metastatic CRPC based on results from the AFFIRM trial, a phase III, randomized, double-blinded study comparing enzalutamide at 160 mg daily with placebo in patients with progressive CRPC who previously received docetaxel chemotherapy[39]. Men were randomized 2:1 to receive enzalutamide vs placebo, and the treatment arm was associated with an improvement of 4.8 months in the primary endpoint of median OS (18.4 vs 13.6 mos, HR 0.63, P<0.001)[39]. FDA approval of enzalutamide was expanded to include chemotherapy-naïve patients in 2014 following analysis of the PREVAIL phase III study comparing enzalutamide with placebo. 1,717 men were randomized 1:1 to enzalutamide vs placebo, with OS and radiographic PFS as co-primary endpoints[40]. Enzalutamide treatment was associated with an 81% reduction in rPFS (65% vs 14%, HR 0.19, P<0.001) and a 29% reduction in risk of death (72% vs 63% survival, HR 0.71, 95% CI 0.60-0.84, P<0.001)[40].

ARN-509

ARN-509 is an AR signaling inhibitor with similar properties to enzalutamide, but with characteristics observed in pre-clinical studies that may predict a higher therapeutic index in patients[41]. ARN-509 is under investigation in several clinical trials, including a multicenter, randomized, double-blind, placebo-controlled, phase III study in men with non-metastatic (M0) CRPC (clinicaltrials.gov: NCT01946204).

TOK-001

TOK-001 (galeterone) is a CYP17 inhibitor that can also down-regulate and antagonize AR [35,42]. In pre-clinical studies, TOK-001 bound wild-type AR with higher affinity than bicalutamide. Unlike bicalutamide, TOK-001 treatment also decreased AR levels by enhancing AR degradation [42]. TOK-001 is currently being investigated in a phase II clinical trial enrolling patients with CRPC (clinicaltrials.gov: NCT01709734).

Targeting microtubules in the treatment of metastatic CRPC

Microtubules are dynamic polymers comprised of α- and β-tubulin heterodimers that are essential for the regulation of the mitotic spindle and chromosomal segregation during cell division[9]. Agents that disrupt microtubule dynamics can therefore serve as effective chemotherapies. Anti-microtubule drugs are of particular relevance in the treatment of prostate cancer. In addition to disrupting mitosis and cell division, microtubule-targeted therapies can also interfere with androgen signaling (Figure 3)[9].

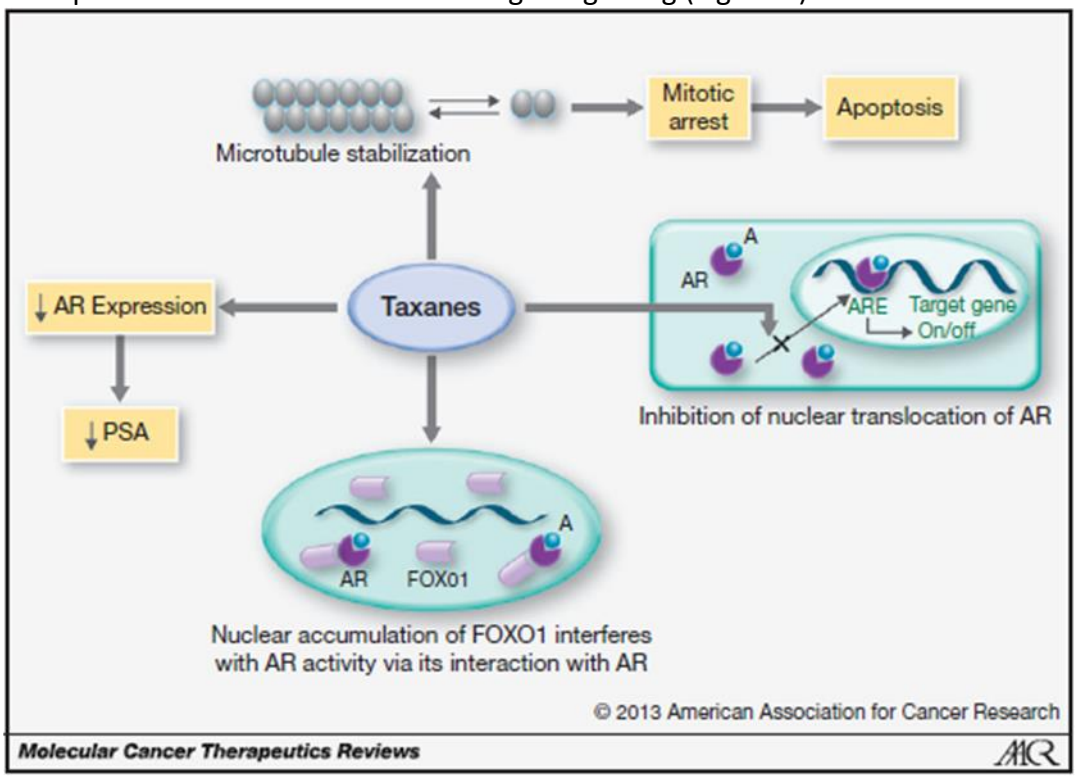

Figure 3. Targeting microtubules in the treatment of prostate cancer. Taxanes may impede prostate cancer progression through multiple mechanisms, including impaired mitotic spindle formation leading to mitotic arrest, inhibition of nuclear translocation of AR, downregulation of AR expression, or induced nuclear accumulation of FOXO1, which suppresses AR activity. From Mistry SJ and WK Oh. (2013) Mol Cancer Ther; 12:555-66.

Microtubules have been shown to play a role in nuclear trafficking of transcription factors, including p53, hypoxia-inducible factor 1 (HIF-1), and AR[9]. Taxane chemotherapies, which are microtubule stabilizers, can decrease AR nuclear accumulation and increase nuclear accumulation of the AR-suppressive FOXO1 protein[9]. Additionally, the taxane docetaxel has been shown to down-regulate AR expression in prostate cancer cell lines[9,43]. The combined effects of impairing mitosis and endocrine signaling may contribute to the particular relevance of taxane chemotherapy in the treatment of metastatic prostate cancer.

Chemotherapy in the treatment of prostate cancer: the role of taxanes

Prior to 2004, no cytotoxic chemotherapy had been shown to prolong life for patients with prostate cancer. This lack of efficacy was felt to be due at least in part to the slow growing nature of prostate cancer in many patients. Evaluation of CRPC metastases showed that prostate cancer cells can have long doubling times (126 +/- 21 days for cancer cells derived from lymph nodes and 94 +/- 15 days for CRPC cells from bone metastases)[9,44]. These data supported the apparent lack of efficacy for chemotherapy against prostate cancer. Indeed, FDA approval of mitoxantrone, a type II topoisomerase inhibitor, and estramustine, a nitrogen-mustard-estradiol conjugate with anti-microtubule and hormonal activity, was granted based on improvements in palliation and treatment responses, respectively, and not on statistically significant improvements in overall survival[45]. However, the activity seen with estramustine prompted subsequent studies in combination with other cytotoxic chemotherapies, particularly additional anti-microtubule agents (docetaxel, paclitaxel, and ixabepilone)[46]. No individual randomized trial of estramustine plus additional chemotherapy was powered to detect an improvement in median OS < 50%[46]. Two subsequent meta-analyses of the activity of estramustine in combination with additional chemotherapy yielded conflicting results, with one identifying an estimated 9.5% increase in OS at 1 year with the addition of estramustine, while the other showed no improvement in overall survival[46,47]. Both meta-analyses evaluated 7 randomized trials, but the former included only the 5 studies for which individual patient data were available, while the latter incorporated all 7 in the analysis[46,47]. Both analyses revealed a marked increase in risk of grade 3 and 4 adverse events with the addition of estramustine, and in particular an associated increased risk of thromboembolic events[46,47].

Docetaxel

The relevance of anti-microtubule agents to the treatment of prostate cancer was reinforced with the study of taxanes for patients with metastatic CRPC. Two randomized phase III trials reported in 2004 demonstrated the clinical efficacy of docetaxel plus prednisone in the treatment of patients with metastatic CRPC. The TAX327 study randomized 1006 men with metastatic CRPC to receive prednisone 5 mg bid with docetaxel 75 mg/m2 every 3 weeks vs 30 mg/m2 weekly for 5/6 weeks vs mitoxantrone 12 mg/m2 every 3 weeks[48]. Men in the every 3 week docetaxel arm experienced an improvement in the primary endpoint of overall survival compared to mitoxantrone (median OS: 18.9 mos vs 17.4 mos for weekly docetaxel vs 16.5 mos for mitoxantrone), and both docetaxel-containing arms resulted in significant improvements in PSA response rate and quality of life ratings[48]. The SWOG 99-16 trial randomized 770 men to 21-day cycles of 280 mg estramustine three times daily on days 1-5 and 60 mg/m2 docetaxel on day 2 with 60 mg dexamethasone in 3 divided doses pre-docetaxel infusion vs. mitoxantrone 12 mg/m2 on day 1 plus prednisone 5 mg twice daily[49]. SWOG 99-16 also showed an improvement in median overall survival for the docetaxel-containing arm (median OS 17.5 vs 15.6 mos, HR 0.80, P=0.02), as well as statistically-significant improvements in time to progression and PSA response rate[49].

Cabazitaxel

Cabaxitaxel is a derivative of docetaxel with decreased affinity for the P-glycoprotein drug efflux pump and improved ability to cross the blood-brain barrier[9]. The TROPIC phase III trial randomized 755 men with metastatic CRPC following docetaxel treatment to receive

prednisone 10 mg daily with cabazitaxel 25 mg/m2 every 3 weeks vs mitoxantrone 12 mg/m2 every 3 weeks[50]. Cabazitaxel treatment was associated with a statistically significant improvement in the primary endpoint of overall survival (15.1 mos vs 12.7 mos, HR 0.70 (95% CI 0.59-0.83, p<0.001), though 82% of men in the cabazitaxel arm experienced grade $\geq$ 3 neutropenia, necessitating the administration of growth factor support[50]. With approval of cabaxitaxel for the treatment of patients with docetaxel-refractory metastatic CRPC, the FDA mandated two additional phase III studies that are ongoing: an open-label non-inferiority study (PROSELICA) of cabazitaxel at 20 mg/m2 vs 25 mg/m2 due to the high rate of febrile neutropenia observed in the TROPIC study, and a three-arm comparison study (FIRSTANA) of cabazitaxel 20 mg/m2 or 25 mg/m2 every 3 weeks vs docetaxel as first-line chemotherapy treatment for metastatic CRPC[51].

Immunotherapy and metastatic CRPC

Immune escape is a hallmark of cancer, with increased activity of immunosuppressive T regulatory cells (Tregs) and myeloid-derived suppressor cells (MDSCs), upregulation of T-cell inhibitory checkpoint pathways via cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death 1 (PD-1), and impaired tumor antigen processing and presentation by antigen presenting cells (APCs) (reviewed in[52]). Prostate cancer cells express specific antigens that are otherwise non-essential and therefore provide attractive targets for therapeutic intervention.

Sipuleucel-T

Sipuleucel-T is an active cellular immunotherapy approved for the treatment of asymptomatic or minimally symptomatic men with metastatic CRPC. It is essentially a vaccination against prostate cancer cells. CD45+ APCs are collected by leukapheresis and pulsed with a fusion construct of the prostate antigen prostatic acid phosphatase (PAP) and granulocyte-macrophage colony-stimulating factor (GM-CSF) called PA2024[52]. Patients undergo three leukapheresis procedures each separated by 2 weeks, with reinfusion of sipuleucel-T three days after each leukapheresis. The road to FDA approval of sipuleucel-T required persistence and the execution of three phase III studies. Two “identically designed” phase III, double-blind, placebo-controlled trials (D9901, D9902A) with time to progression (TTP) as the primary endpoint enrolled 225 patients (147 sipuleucel-T vs 78 placebo) and failed to demonstrate improvement in the primary endpoint (TTP), but showed apparent improvement (D9901) and trend toward improvement (D9902A) in median OS[53,54]. This unexpected result led to a third randomized controlled trial (IMPACT) with OS as primary endpoint. Men with asymptomatic or minimally symptomatic metastatic CRPC were randomized 2:1 (n = 512) to receive sipuleucel-T or APCs not pulsed with PA2024[55]. Approximately 85% of subjects were chemotherapy-naïve. Sipuleucel-T was associated with an improved median OS (25.8 vs 21.7 mos, HR 0.77; P = 0.2) despite permitted crossover to allow men in the “placebo” arm to receive frozen / stored sipuleucel-T (63.7%)[55]. Interestingly, there was no delay in the first TTP, and few patients experienced decline in PSA or objective responses on imaging.

Immunotherapies under investigation in CRPC

ProstVac-VF TRICOM is a poxvirus-based vaccine therapy where recombinant vaccinia and fowlpox vectors express prostate specific antigen (PSA) and costimulatory molecules (TRICOM). Instead of using a patient's own antigen presenting cells, as with sipuleucel-T, ProstVac-VF relies on poxviruses carrying PSA to stimulate an immune response to both the immunogenic viral vaccine itself, and also the tumor antigen[51,52]. Promising clinical activity in phase II studies has led to an ongoing three-arm, placebo-controlled, randomized phase III study (PROSPECT) of ProstVac-VF TRICOM with or without the growth factor GM-CSF in men with asymptomatic or minimally-symptomatic metastatic CRPC (clinicaltrials.gov: NCT01322490) [51,52].

Ipilimumab, a monoclonal antibody targeting the immunosuppressive cytoxic T-lymphocyte antigen-4 (CTLA-4), is approved for the treatment of patients with metastatic melanoma. A randomized phase III trial comparing ipilimumab to placebo following a single fraction of radiation therapy to bone lesions in the treatment of men with metastatic CRPC who had undergone prior docetaxel treatment failed to demonstrate a statistically significant survival benefit with use administration of ipilumimab at 10 mg/kg (HR 0.70; 95% CI, 0.61-1.00; p = 0.053), but showed an improvement in progression-free survival (HR 0.70; 95% CI, 0.61-0.82; p < 0.0001)[51]. An ongoing randomized, double-blind, phase III study is evaluating ipilumimab vs placebo in the treatment of men with asymptomatic or minimally-symptomatic chemotherapy-naïve metastatic CRPC (clinicaltrials.gov: NCT01057810).

The bone microenvironment as a therapeutic target for metastatic CRPC

Prostate cancer is especially bone-tropic (Figure 4A). Approximately 90% of patients with metastatic prostate cancer have bone metastases[56]. The mechanisms by which prostate cancer cells home to bone and thrive there likely involve the interplay of chemotactic [eg. stromal-derived factor 1 (SDF-1)], angiogenic, adhesion (eg. cell adhesion molecules preferentially expressed by human bone marrow endothelial cells and collagen type I in bone matrix), and growth factors [eg. insulin-like growth factor (IGF-1), fibroblast growth factors (FGF), bone morphogenic proteins (BMP), and transforming growth

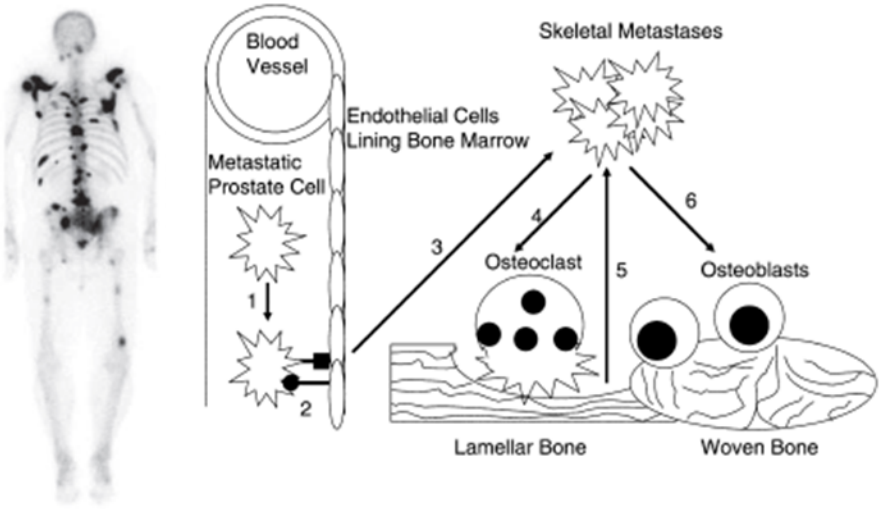

Figure 4. Prostate cancer is bone-tropic. A. Bone scan from a patient with metastatic CRPC. B. Model of cross-talk between prostate cancer and bone microenvironment. From (A) Smith DC, et al. (2013) J Clin Oncol; 31:412-19. (B) Keller ET, et al. (2001) Cancer Metast Rev; 333-49.

factor-β (TGF-β)] produced by the bone microenvironment (reviewed in[56,57]). Upon reaching bone, prostate cancer cells produce factors that stimulate osteoclasts that break down bone matrix, causing release of growth factors from bone matrix to further support prostate cancer cell proliferation (Figure 4B)[56,57]. The developing osseous metastases release factors that support osteoblast proliferation and survival[56,57]. Both the bisphosphonate zoledronic acid and the RANKL inhibitor denosumab work to impair osteoclastogenesis and have been approved for use in reducing skeletal-related adverse events (SREs) in patients with metastatic CRPC.

Targeting prostate cancer bone metastases

Radium-223 is a radioactive calcium mimic that undergoes preferential uptake in rapidly growing bone at the sites of osseous metastases compared to normal bone[45]. The clinical utility of radium-223 derives from its predominant α-decay[58]. Alpha particles are effective at inducing DNA double-strand breaks and emit over a shorter path-length than lower-energy β particles[45]. Unlike Strontium-89 and samarium-153 lexidronam, which are predominantly beta-emitters approved to provide palliation for painful bone metastases, radium-223 has been associated with improvement in overall survival for patients with metastatic CRPC. The phase III ALSYMPCA study randomized 921 patients with metastatic CRPC with symptomatic bone metastases previously treated with, unfit for, or refusing docetaxel in a 2:1 fashion to receive 6 injections of 50 kBq/kg radium-223 every 4 weeks vs placebo[59]. Eligible patients had > 2 bone lesions and no visceral metastases or bulky (>3 cm) lymph nodes. Radium-223 was associated with an improvement in median OS (14.9 vs 11.3 mos, HR 0.70, 95% CI 0.58-0.83, P<0.001) and prolonged time to first SRE (15.6 vs 9.8 mos, HR 0.66, 95% CI 0.52-0.83, P<0.001)[59].

Ongoing and future investigations in the treatment of metastatic prostate cancer

Several questions remain regarding the use of novel second-line hormonal therapies in the treatment of CRPC and their sequencing or combination with immunotherapies, chemotherapies, or other targeted therapies. Optimal timing and sequencing of these agents remains unknown. We know that prior therapy influences response to subsequent anti-cancer regimens. Should CYP17 inhibitors or second-generation AR signaling inhibitors be used prior to chemotherapy, or prior to sipuleucel-T? Should CYP17 inhibition and / or AR blockade be maintained following disease progression, just as castration therapy is continued in the setting of CRPC? Several ongoing clinical trials are attempting to address questions of optimal sequencing and combination of approved therapies for CRPC.

Emerging therapeutic approaches: exploiting mechanisms of resistance to androgen CYP17 and AR signaling inhibitors

Ongoing and future studies also will investigate the safety and efficacy of combinations of second-line hormonal therapies, for example the combined use of a CYP17 inhibitor, AR signaling inhibitor, and the 5α-reductase inhibitor dutasteride. Studies of potential mechanisms of resistance to CYP17 inhibition support the rationale for combination therapy [60,61]. In *in vitro* models, treatment with abiraterone acetate can result in increased CYP17A1 expression, increased AR expression, and increased expression of AR splice variants that are ligand-

independent [60,61]. The T877A mutant AR found in some CRPC is not CYP17A1 dependent, but does depend on CYP11A1[60]. In metastatic tumor biopsies from patients with CRPC with progressive disease on treatment with a CYP17A1 inhibitor (abiraterone acetate or ketoconazole), selection for tumor cells expressing progesterone-activated mutant ARs was identified as a mechanism of resistance to CYP17A1 inhibition[62].

Other enzymes in the androgen biosynthetic pathway are emerging as viable targets for inhibition (Figure 2). Aldo-keto reductase 1C3 (AKR1C3) catalyzes the final step in testosterone synthesis [63]. AKR1C3 is overexpressed in CRPC, and potent, selective inhibitors of AKR1C3 have been generated [63]. Additionally, Sharifi and colleagues identified an alternative pathway to DHT production that bypasses testosterone that may be involved in the development of CRPC [64]. They also showed that a mutation that stabilizes 3βHSD, the enzyme that catalyzes the conversion of dehydroepiandrosterone (DHEA) to androstenedione, the rate limiting step in this pathway upstream of DHT, is associated with CRPC and may provide a mechanism for abiraterone resistance[65]. These data suggest that 3βHSD may also serve as a potential therapeutic target in CRPC.

Combining maximal androgen synthesis inhibition through castration therapy plus CYP17 inhibition with AR blockade is also being investigated in patients with metastatic CRPC. However, glucocorticoid receptor upregulation is a mechanism of acquired resistance to AR inhibition by enzalutamide[66]. It is therefore possible that combining CYP17 inhibition, which requires steroid supplementation to offset toxicities related to loss of negative feedback control of ACTH, with enzalutamide or other second-generation anti-androgens, will not improve outcomes compared to sequential therapy.

Additionally, alterations in AR structure are associated with resistance to CYP17 inhibition and to clinically available anti-androgens that target the C-terminal ligand binding domain of AR. The androgen receptor is comprised of an N-terminal domain, a DNA-binding domain, and a C-terminal ligand-binding domain. Abiraterone can induce expression of full-length AR and AR splice variants that are capable of ligand-independent transactivation, leading to abiraterone resistance[61]. The presence of the AR-V7 splice variant, which lacks the ligand-binding domain, in circulating tumor cells from patients with metastatic CRPC appears to be associated with resistance to enzalutamide and abiraterone[67]. Small molecule antagonists of the AR N-terminal domain and peptidomimetics that disrupt AR-coregulatory protein interactions have demonstrated activity in pre-clinical studies of CRPC and are examples that may point the way to future therapeutic approaches for enzalutamide and abiraterone-resistant prostate cancer[68,69].

Targeting alternative oncogenic signaling pathways in CRPC

Several oncogenic pathways are relevant to the development and progression of CRPC in addition to androgen signaling (Figure 5). Some are in early stages of clinical investigation, while others recently have fallen short of expectations when subjected to the rigor of phase III trials. Select pertinent examples are discussed below.

Cabozantinib is a small molecule inhibitor of the c-Met and VEGFR2 receptor tyrosine kinases that can contribute to proliferation, metastasis, and angiogenesis. Cabozantinib is approved for the treatment of metastatic medullary thyroid cancer based on improvement in PFS[70]. A phase II multi-national randomized discontinuation trial in 9 tumor types, including mCRPC, and a subsequent open-label phase II expansion study in men with chemotherapy-pretreated metastatic CRPC showed that cabozantinib was associated with pain relief and particularly dramatic responses in osseous metastases[71,72]. These studies generated excitement over the potential efficacy of cabozantinib for patients with CRPC. However, a randomized, double-blind phase III trial comparing cabozantinib with prednisone in men with bone-predominant metastatic CRPC who had progressed following prior treatment with docetaxel and abiraterone or enzalutamide failed to demonstrate a statistically significant improvement in median OS (http://onclive.com, published online 9/2/14, accessed 11/30/14).

Clusterin is an anti-apoptotic protein associated with activation of oncogenic, pro-metastatic, and angiogenic signaling[73]. Clusterin is upregulated by androgen deprivation therapy, radiation therapy, and chemotherapy[73]. A randomized phase II study of custirsen, an antisense to clustiren, demonstrated that addition of custirsen to prednisone and either docetaxel or mitoxantrone in the treatment of men with metastatic CRPC progressing during or within 6 months of docetaxel treatment was associated with a high rate of durable pain responses[74]. A phase III randomized trial (SYNERGY) that enrolled 1022 men with metastatic CRPC failed to show a significant survival benefit with the addition of custirsen to docetaxel plus prednisone as first-line treatment (http://onclive.com, published online 4/28/14, accessed 11/30/14). The AFFINITY study, a randomized phase III trial of cabazitaxel plus prednisone with or without custirsen as second line chemotherapy for men with metastatic CRPC, has completed enrollment (clinicaltrials.gov: NCT01578655).

Enzymes within the phosphoinositide 3-kinase (PI3K) signaling cascade also provide attractive potential targets in the treatment of CRPC. PI3K signaling is critical for regulation of cell growth, proliferation, and survival; angiogenesis; and insulin signaling[75]. The PI3K pathway is frequently altered in prostate cancer, with 100% of patients with metastatic prostate cancer demonstrating alterations in components of the pathway in one series[76]. Loss or functional impairment of the tumor suppressor phosphatase and tensin homolog (PTEN), a lipid and protein phosphatase that antagonizes PI3K signaling, is frequent in CRPC and correlates with poor disease-specific mortality[77]. PTEN loss is associated with repression of AR responsive genes, while inhibition of PI3K signaling activates AR signaling through loss of feedback inhibition of HER family receptor tyrosine kinases[78]. This reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN-deficient prostate cancer raises the possibility that dual inhibition of PI3K signaling and and AR may be clinically relevant. Combined inhibition has resulted in PTEN-deficient tumor regression in pre-clinical studies[78]. Phase II studies combining rapamycin analogues everolimus or deforolimus, inhibitors of mammalian target of rapamycin (mTOR) in the PI3K pathway, with the first-generation anti-androgen bicalutamide have shown underwhelming clinical activity for these combinations[79,80]. However, combination studies with second generation antiandrogens, abiraterone, and novel inhibitors of PI3K itself are underway and may yield more promising results.

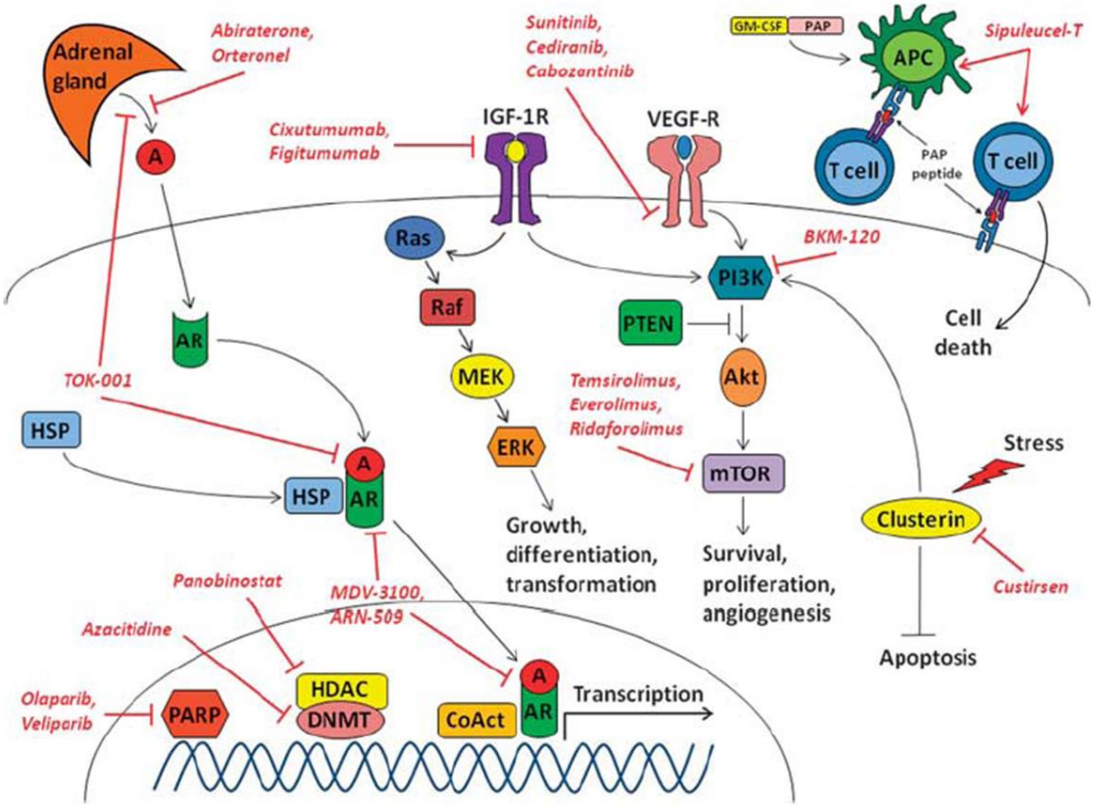

Figure 5. Potential therapeutics targets in CRPC. (MDV3100 is enzalutamide.) From ES Antonarakis and AJ Armstrong. Prostate Cancer and Prostatic Diseases 2011; 14:206–218.

Introducing agents effective against CRPC earlier in the treatment of prostate cancer

It is possible that the best opportunities for achieving durable responses for patients with newly-diagnosed metastatic castration-sensitive prostate cancer will arise from ongoing and future studies that seek to move therapies targeting nodes of resistance earlier in the disease process. The recently reported CHAARTED study of ADT +/- docetaxel and prednisone for men with metastatic castration-sensitive prostate cancer exemplifies this approach.

The concept of treating castration-sensitive prostate cancer with an aggressive combination of ADT and chemotherapy is not new. However, until recently clinical evidence to support this approach was lacking. A previous phase III trial (GETUG-AFU 15) of 385 men with metastatic castration-sensitive prostate cancer randomized in a 1:1 fashion to standard treatment with ADT (orchiectomy or LHRH agonist with or without non-steroidal anti-androgen therapy) or ADT plus up to 9 cycles of docetaxel (75 mg/m2 every 21 days) failed to demonstrate a survival benefit for the addition of docetaxel (median OS 58.9 vs 54.2 months, HR 1.01, 95% CI 0.75–1.36), and the addition of docetaxel was associated with 72 serious adverse events (SAEs) compared to none with ADT alone[81]. Approximately 22% of these men had “high tumor burden” at baseline, and 62% randomized to ADT alone subsequently went on to receive

docetaxel[81,82]. This study appeared to confirm the notion that taxanes should not be used prior to the development of castration resistance.

The ChemoHormonal Therapy vs Androgen Ablation Randomized Trial for Extensive Disease in Prostate Cancer (CHAARTED) was designed to assess the impact of addition of up to 6 cycles of docetaxel to ADT in men with high-volume metastatic prostate cancer, and was subsequently amended to include men with lower volume disease to facilitate accrual. Of 790 men randomized 1:1 to either treatment arm, 64% of men treated with ADT alone and 67% treated with ADT + docetaxel had "high volume" disease, defined as visceral disease and/or 4 or more bone metastases with extra-axial bone involvement[82]. Introduction of docetaxel within 4 months of initiation of ADT resulted in a 13.6 month improvement in overall survival (57.6 vs 44.0 months, HR=0.61 (0.47-0.80), p=0.003[82]. There was one treatment-related death in the ADT + docetaxel arm. There were no deaths attributed to ADT alone. Unlike GETUG-AFU 15, the majority of men (67%) randomized to the ADT alone arm in the CHAARTED study did not go on to receive future docetaxel therapy, suggesting that men with a higher disease burden at diagnosis may not tolerate delayed chemotherapy treatment[81,82]. On subset analysis, the survival benefit observed with addition of docetaxel in the CHAARTED study was most dramatic for men with high-volume disease, who experienced a 17.7 month improvement in median OS when compared to those who received ADT alone [49.2 vs 33.2 months, HR 0.60 (0.45-0.81), p=0.006][82]. In the smaller population who had a more limited extent of disease / no visceral metastases, the median OS had not yet been reached in either arm at the time of analysis. Based on these results, addition of up to 6 cycles of docetaxel to ADT should be considered for eligible patients with high-volume castration-sensitive prostate cancer. Whether men with less extensive disease burden should receive up-front chemotherapy remains to be determined.

Whether additional agents with demonstrated activity against CRPC, including androgen signaling inhibitors, immunotherapy, or bone targeted therapies will benefit patients earlier in the disease process, either in the setting of locally advanced disease, castration-sensitive metastatic disease, or non-metastatic CRPC, remains to be determined. Several studies are underway seeking to address these questions.

Conclusions

The therapeutic landscape for metastatic prostate cancer has expanded dramatically in the past four years, owing to our growing understanding of the biology underlying prostate cancer progression and resistance to treatment. These recent advances clearly demonstrate that androgen signaling remains important and sensitive to effective therapy in what was once considered to be "androgen independent" disease. Further evaluation of the safety and efficacy of combination therapies that target multiple mechanisms of progression and resistance, including androgen signaling inhibitors, anti-microtubule agents, immunotherapies, bone-targeted therapies, and inhibitors of alternative oncogenic signaling pathways is anticipated to yield further advances in the treatment of metastatic prostate cancer in the near future.

Key points:

- "Castration resistant" is not equivalent to "androgen independent"
- Abiraterone acetate, an inhibitor of CYP17 involved in androgen synthesis, is associated with improved overall survival in patients with chemotherapy-naïve or docetaxel-refractory metastatic CRPC
- Enzalutamide, an AR signaling inhibitor, is associated with improved overall survival in patients with chemotherapy-naïve or docetaxel-refractory metastatic CRPC
- Docetaxel and cabazitaxel are microtubule stabilizers that are associated with improved overall survival for patients with metastatic CRPC
- Immunotherapies are under active investigation in the treatment of metastatic CRPC, and the cell-based immunotherapy sipuleucel-T is associated with improvement in overall survival in asymptomatic or minimally symptomatic patients
- Radium-223 is a predominantly α-emitting radiopharmaceutical which has improved overall survival in men with painful bone-predominant metastatic CRPC
- The optimal sequencing and combination of approved agents remains unknown
- The role for approved and investigational therapies for "castration resistant" prostate cancer earlier in the disease course (localized or "castration sensitive" metastatic disease) is being investigated

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