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New Molecular Targets in Metastatic Prostate Cancer

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Abstract

Prostate cancer (PCa) is the second leading cause of cancer death in men. Despite initial responses, almost all patients progress to castration-resistant prostate cancer (CRPC). Over the past decade, increased understanding of the mechanisms that drive resistance to castration has led to the development of next-generation androgen receptor targeting agents such as abiraterone acetate and enzalutamide. Moreover in the last few years, results from large Phase III trials led to the approval of an α-emitter (radium-223), the bone resorption-targeting drug denosumab and an immunotherapy (sipuleucel-T) that showed improvements in terms of overall survival. In the field of metastatic CRPC, other novel therapeutics have recently been proven to extend survival via distinct mechanisms of action such as the new and more potent classes of androgen inhibitors, ortonel, ARN-509 and galeterone, the endothelin A receptor antagonist zibotentan, the Src inhibitor dasatinib, the c-MET inhibitor cabozantinib and the immune checkpoint inhibitor ipilimumab. This review aims to revisit the evolution of androgen receptor targeting therapeutics and to discuss other important alternative biologic pathways that have given rise to new agents in metastatic prostate cancer.

Keywords

Prostate Cancer, Target Therapy

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1. Introduction

Prostate cancer (PCa) is currently the second leading cause of cancer-related deaths in men in the United States and the most common cancer in elderly males in Europe [1] [2]. The main predictors of prostate cancer risk are age, race or ethnicity, and family history. There are numerous molecular, genetic, environmental, and dietary factors, with varying degrees of supporting evidence. Although prostate cancer typically manifests in old age, a growing body of evidence suggests that prostatic carcinogenesis is initiated much earlier. Prostatic intraepithelial neoplasia (PIN) is the histologic entity widely considered to be the most likely precursor of invasive prostate cancer. Although not all patients with high-grade PIN (HGPIN) progress to develop invasive disease, PIN is characterized by cellular proliferation within pre-existing ducts and glands, with cytologic changes that mimic those of cancer [3]. PIN is associated with progressive abnormalities of phenotype and genotype that are intermediate between normal prostatic epithelium and cancer [3]. The majority of men diagnosed with prostate cancer will benefit from not being treated, because they have low-volume indolent tumors that do not require immediate treatment. Active surveillance is a reasonable and widely accepted approach for these patients [4]. On the other hand localized PCa is the category of men presenting either with a high-risk localized cancer or with metastatic disease that are usually treated aggressively with prostatectomy, radiation therapy and/or androgen deprivation therapies (ADT). ADT is the standard of care for advanced prostate cancer. This treatment drives to prostate-specific antigen (PSA) decrease and clinical improvements in more than 90% of patients [5]. Nevertheless, this therapeutic approach is not curative and the majority of patients often develop castration resistance. Moreover, most men with advanced prostate cancer are at high risk for developing bone metastases [6]. Bone metastases are associated with a reduced quality of life and an increased risk of complications such as pathological fractures, spinal cord compression, radiation or surgery to the bone that are collectively defined as skeletal-related events (SREs) [7].

In the last years, the introduction of highly effective novel treatments has significantly changed the management of metastatic castration resistant prostate cancer (mCRPC) patients with an improved overall survival (OS) advantage [8]. These agents include a chemotherapeutic (cabazitaxel), two hormonal agents (abiraterone and enzalutamide), an alpha-emitting bone-seeking radioisotope (radium-223) and an immunotherapeutic agent (sipuleucel-T). In particular in 2011, the novel tubulin-binding taxane, cabazitaxel, as a second-line chemotherapy showed encouraging results [9]. Between 2011 and 2012, abiraterone acetate and enzalutamide showed further OS improvements [10] [11] as well as in 2013, radium-223 became available in clinical practice [12]. In 2011 it was demonstrated that treatment with denosumab significantly prolonged the median time to the first SRE compared to zoledronic acid in CRPC patients suffering with bone metastases [13]. Finally, immunotherapy with sipuleucel-T, an immune-modulating agent (FDA approved in 2010) showed a survival advantage [14], but it was not widely used because, rarely, patients responded to therapy.

Advances in the understanding of the biology of metastatic PCa have led to major improvements in the area of targeted therapies, especially in recent years. Indeed, novel agents now in clinical evaluation include AR inhibitors (ARN509, ODM-201), new generation hormonal therapies (galeterone, orteronel) immunotherapies (like ipilimumab) and drugs targeting key molecular pathways involved in tumor progression and bone metastases onset (cabozantinib, dasatinib, saracatinib, zibotentan).

This review provides an overview of the current therapeutic options for the treatment of mCRPC, including novel agents currently in clinical evaluation.

2. Targeting the Androgen Pathway

Recent evidence has demonstrated that androgen-based pathways remain a key signal in the progression of CRPC. Indeed, tumor tissues express several enzymes that regulate the synthesis of testosterone and dihydrotestosterone, such as cytocrome P450 17 alpha hydroxysteroid dehydrogenase (CYP17) [15]. Persistent androgen signaling represents an effective therapeutic target in mCRPC.

Several preclinical and clinical data confirmed that the shift from endocrine-dependent to intracrine androgen signaling progression is essential for the progression of prostate cancer and for the resistance to main androgen deprivation treatments [16] [17]. Furthermore during mCRPC progression, androgen receptor (AR) undergoes molecular changes, such as overexpression, mutation, alternative splicing, post-translational modifications and interactions with other pathways (non-classical AR signaling) [18] [19].

2.1. Abiraterone

Abiraterone acetate is an orally administered steroidal antiandrogen derived from the structure of pregnenolone. It inhibits androgen biosynthesis blocking the hydroxylase and lyase activities of CYP17A with 10 - 30 fold stronger than ketoconazole [20]. Consequently, serum and intratumoral androgen production in the adrenal gland, testes and prostate cancer cells became undetectable [21] [22]. Abiraterone is usually administered with prednisone to ameliorate the secondary increase of the adrenocorticotropic hormone that can lead to excess mineralocorticoid synthesis [23].

Two randomized Phase III trials demonstrated that abiraterone improved OS compared with placebo. In the COU-AA-301 trial [10], 1195 patients previously treated with docetaxel were randomized to abiraterone plus prednisone or placebo plus prednisone. The primary endpoint of OS was met with an improvement in OS of 3.9 months compared with placebo. In the COU-AA-302 trial, 1088 chemotherapy-naïve patients were randomized to abiraterone plus prednisone or to placebo plus prednisone. The results showed an 8.2-month improvement in radiographic progression free survival (PFS) favoring abiraterone. Abiraterone treatment was also associated with better pain control from skeletal metastases, a delay in development of SREs, and delayed radiological skeletal progression. More specifically, 25% of patients developed a skeletal event in 9.9 months when treated with abiraterone versus 4.9 months with placebo, and the time to first SRE was 25.0 months with abiraterone compared to 20.3 months with placebo [24] [25]. Abiraterone was well tolerated in this trial although adverse effects due to increased mineralocorticoid levels secondary to CYP17 blockade were more common with abiraterone. Specific effects that were significantly higher with abiraterone than placebo included fluid retention and edema (31% vs 22%) and hypokalemia (17% vs 8%) [25]. These abiraterone benefits on metastatic bone disease may be attributable not only to a direct antitumor effect, but also to specific action on the bone microenvironment. Indeed, recently a direct anabolic and an anti-reabsorptive effect on bone by abiraterone, both in vitro and in mCRPC patients was found. Our research team demonstrated that abiraterone was able to specifically modulate osteoclasts and osteoblasts leading to direct anabolic and anti-reabsorptive effects both in presence and absence of steroids, suggesting a non-canonical mechanism of action that seems to be, at least in part, androgen-independent [26].

2.2. Enzalutamide

Enzalutamide is another promising oral AR inhibitor that targets multiple steps in the AR signaling pathway. Two Phase III trials have demonstrated the efficacy of enzalutamide in the treatment of patients with mCRPC.

A randomized Phase III trial (AFFIRM) showed that mCRPC patients treated with enzalutamide after docetaxel had a significant improvements in OS compared to placebo group. Further benefits concerned the delay in time to first SRE and improvements in bone pain and quality of life [11].

Moreover, in the Phase III PREVAIL study evaluating enzalutamide versus placebo in patients with mCRPC, who had not received chemotherapy, the anti-androgen significantly reduced the risk of bone disease progression and death. The study also showed significant improvements in other secondary and prespecified exploratory end points, such as delayed start of chemotherapy, lower risk of first SRE and an increase of the percentage of responded compared with placebo [27]. Moreover enzalutamide is reported to cause fatigue (11%), hot flashes (20%), headache (12%), nausea, diarrhea, constipation and musculoskeletal pain [11].

Ongoing trials could further define the optimal use of abiraterone acetate in combination with enzalutamide for treating mCRPC patients.

2.3. Orteronel

Orteronel (TAK-700), like abiraterone, is an androgen production inhibitor with a selectivity for 17 - 20 lyase over 17-α-hydroxylase, but with fewer mineralcorticoid effects. Moreover, for long-term therapy orteronel could be administrated with prednisone [27].

A Phase I/II open-label study in patients with mCRPC showed that orteronel treatment decreased PSA, testosterone and dehydroepiandrosterone levels [28]. The majority of side effects were lower grade even if some high-grade events were reported such as hypertension (in 13% of patients), shortness of breath (in 8%), and pneumonitis (in 5%) [28].

Currently, two ongoing randomized, placebo-controlled Phase III trials are assessing orteronel in patients with

progressive CRPC who are either chemotherapy naive or pretreated with docetaxel. Unfortunately no improvement in OS was observed in treated patients compared with placebo, although the treatment led to longer relative PFS [29].

2.4. Galeterone

Galeterone (TOK-001) is an androgen biosynthesis inhibitor with a biological effect that changes with its concentration: at low concentrations, it blocks CYP17A1 activity; at moderate concentrations, it acts as an AR antagonist; and at high concentrations, it drives to AR degradation [26]. Based on phase I trial (ARMOR1) data, galeterone received fast-track designation from the FDA for the treatment of mCRPC [30]. Most side effects were minor and included fatigue, nausea and diarrhea, but nothing requiring cortisol treatment [30]. The Phase II ARMOR2 trial in 25 progressive CRPC patients confirmed safety and efficacy and showed a 4.8-month median OS benefit compared with placebo [31].

2.5. ARN-509

ARN-509 is a second generation AR inhibitor that binds to AR preventing growth and androgen-mediated gene transcription *in vitro*, through the inhibition of AR nuclear translocation and its consequent DNA bond [32]. Phase I clinical trials demonstrated that ARN-509 decreased PSA levels of ≥50% from baseline at 12 weeks in 46.7% of treated patients. The toxicity profile included fatigue (38%), nausea (29%) and pain (24%). It is currently being evaluated in Phase II clinical trials and published data shows a significant PSA response [33].

2.6. ODM-201

ODM-201 is another promising prostate cancer treatment that, with its major metabolite, ORM 15341, specifically inhibits AR nuclear translocation [34]. ODM-201 showed a higher anticancer activity compared with enzalutamide in xenograft models of prostate cancer.

In a Phase II randomized trial, 124 treated patients displayed a clinical benefit in terms of overall response rate and bone response [35]. The most common treatment side events were fatigue or asthenia (12%), hot flush (5%), and decreased appetite (4%).

3. Targeting Bone Microenvironment

Novel therapies in the management of prostate cancer target both the cancer cells as well as the bone microenvironment, preferential site of prostate cancer metastases.

Prostate bone metastases are typically osteoblastic, thus characterized by both an osteoblastic proliferation with enhanced matrix deposition and an increased osteoclastic activity [36] [37]. The result is an increase of osteoblast proliferation and differentiation which increase the deposition of abnormal woven bone [38]. Enhanced osteolytic activity causes the release of growth factors stored in the bone matrix into the tumor microenvironment that stimulate tumor cell growth and alter their phenotype, thus promoting a vicious cycle of metastasis and bone pathology. Increased osteolysis makes SREs a very common feature of bone metastatic prostate cancer patients. SREs are very uncommon when the disease is androgen sensitive, nevertheless their incidence increase when prostate became castration resistant [39].

In this regard, bisphosphonates and denosumab currently represent two effective additional approach in the management of metastatic prostate cancer and several randomized, controlled trials supported their efficacy in reducing skeletal morbidity of patients [40].

3.1. Bisphosphonates

There are two groups of bisphosphonates, non-nitrogen-containing and nitrogen-containing, with different effects on osteoclasts. Etidronate, clodronate and tiludronate belong to the non-nitrogen-containing class of bisphosphonates, whereas pamidronate, alendronate, ibandronate, risedronate and zoledronic acid belong to the nitrogen-containing bisphosphonates (which are more potent osteoclast inhibitors and more commonly used). Bisphosphonates drive to osteoclast apoptosis, affecting their differentiation and maturation and thus inhibiting their bone resorption activity. Moreover bisphosphonates influence macrophages, gamma delta T cells, osteob-

lasts and tumor cells as demonstrated in preclinical models. In addition to their effects on host cells, bisphosphonates could also have antitumor and/or antiangiogenic effects [41].

Zoledronic acid is currently approved for the treatment of patients with bone metastatic prostate cancer that is progressing while on initial hormone therapy. A phase III trial supported the efficacy of zoledronic acid in this subset of patients that after the treatment showed a significant decrease of SREs incidence, a longer median time to develop SREs and better pain scores [42].

Osteoclastic proliferation, differentiation, activation and apoptosis is regulated by RANK (Receptor Activator of Nuclear Factor-kB), RANKL (Receptor Activator of Nuclear Factor-kB Ligand) and the decoy Receptor Osteoprotegerin (OPG). These factors belong to TNF and TNF receptor superfamily and have a central role in the establishment of bone metastases. Indeed RANKL induce osteoclast-mediated bone resorption and the consequent release of matrix growth factors such as Tumor Growth Factor-b (TGF-b) and platelet-derived growth factor (PDGF) that in turn enhance the growth of tumor cells establishing a positive feedback mechanism [43].

Finally, it has been demonstrated that RANK is expressed not only on osteoclasts, but also on prostate cancer cells [44] suggesting that RANK allows cancer cells to migrate where RANKL is abundantly expressed, like the bone.

3.2. Denosumab

Denosumab (AMG162) is a human non-cytotoxic IgG2 monoclonal antibody with an extremely high affinity and specificity for human RANKL. It is approved for the treatment of osteoporosis, cancer treatment induced bone loss, bone metastases and other skeletal pathologies mediated by osteoclasts.

In a castration-resistant prostate cancer population presenting with bone metastases, the median time-to-first SRE for the denosumab arm was significantly prolonged (21 months) compared to the zoledronic acid arm (17 months) with no improvements in OS or progression of disease [13].

Moreover, in a Phase III trial in men with nonmetastatic castration-resistant prostate cancer with a high risk of developing bone metastases, denosumab significantly increased bone-metastasis-free survival by a median of 4 months compared to placebo (29 vs 25 months) potentially confirming the role of RANK/RANKL in regulating cancer cell homing to the bone [13].

3.3. Safety of Bone Target Therapies

One of the most commonly reported adverse event related to bisphosphonates and denosumab treatment is hypocalcaemia that is most often asymptomatic with these agents. In particular, hypocalcaemia occurred more frequently with denosumab than with zoledronic acid as shown in the Phase III trial in patients with CRPC and bone metastases (13% vs 6%) [13]. In an integrated analysis of 5723 patients from three randomized Phase III trials, the safety profile for denosumab was better than for zoledronic acid, demonstrating no effect on renal function and no need for dose adjustment or renal monitoring [45]. In patients receiving zoledronic acid the incidence of hypocalcaemia was lower than in patients receiving denosumab (1.3% vs 3.1% for grade 3 or grade 4 toxicities), though most cases were asymptomatic [45]. Thus, repletion of vitamin D levels before and during the therapy and monitoring of calcium levels during therapy is recommended in the prescribing information of denosumab.

3.4. Radiopharmaceuticals

Radiopharmaceuticals are other interesting agents targeting bone metastases; several studies showed how beta-emitting radiopharmaceuticals allowed bone pain relief in mCRPC patients due to their similarity to calcium, emitting radiation when they were taken up at site of osteoblastic activity.

Stronzium-89 and samarium-153 were the first radiopharmaceuticals approved for bone metastases pain relief in patients with mCRPC [46]. Although these radiopharmaceuticals are useful tool for pain palliations, no study showed impact on OS. One randomized control trial showed that stronzium-89, after six cycles of docetaxel, improved clinical PFS but frequent hematological adverse events [47] limits their use only to symptomatic patients with multiple bone metastases.

Radium-223 is an alpha emitter that differs from beta emitter agents since it delivers a highly localized radiation to the bone surface, causing double-stranded DNA breaks that lead to cell death giving less irradiation to

healthy bone marrow than beta-emitters [48]. It is a calcium mimetic molecule that forms a complex with hydroxyapatite, which forms 50% of bone matrix; this linking allows radium 223 to be incorporated into the bone matrix emitting alpha particle preserving the health of bone tissue and bone marrow and limiting distribution to soft tissue [49].

Radium-223 was recently approved by FDA for men with symptomatic mCRPC with only bone metastases showing a significant impact on OS in patients who progress with docetaxel or unfit to docetaxel. Several Phase I and II trials showed safety and tolerability of alpharadin, radium-223 chloride in solution, in mCRPC patients, with improvements in bone turnover markers such as bone alkaline phosphatase (bALP) and urine N-telopeptide (uNTX) [50] [51]. These results led investigators to conduct a randomized open-label, multicenter Phase III trial evaluating the impact on OS of radium-223 in mCRPC patients with bone metastases previously treated with docetaxel or unfit to receive docetaxel. This Phase III trial was stopped early after pre-planned efficacy interim analysis, since OS was significantly improved in the radium-223 arms versus placebo-control arm (median, 14.0 vs 11.2 months respectively); updated analyses in all 921 patients, performed before crossover from placebo to radium-223, showed a similar survival advantage for radium-223 treatment (median, 14.9 vs 11.3 months) [12]. Moreover, radium-223 showed efficacy in all secondary end points including time to the first symptomatic skeletal events (median, 15.6 months vs 9.8 months, respectively). The side effects of radium 223 can include diarrhoea and sickness but these are generally mild. Starting from these promising results, new trials are under investigation to better understand combination therapy with docetaxel and other new emergent therapies such as abiraterone acetate that will improve OS in this subset of patients.

4. Targeting Signal Transduction Pathways

4.1. Targeting c-MET/HGF Pathway

The receptor tyrosine kinase MET and its ligand hepatocyte growth factor (HGF) signaling pathway promotes stemness phenotype, tumor growth, invasion and metastases in several malignancies.

MET is expressed by primary and metastatic prostate carcinomas and its levels are higher in bone metastases compared with lymph node metastases or primary tumors [52]. Also osteoblasts and osteoclasts express MET and HGF regulating cellular responses, such as proliferation, migration and differentiation.

Cabozantinib (XL184) is an orally tyrosine kinase inhibitor that targets MET and VEGF receptor 2 (VEGFR2). In a multicenter, Phase II, nonrandomized expansion study of men with CRPC, bone metastases and disease progression despite docetaxel treatment, Cabozantinib was associated with improvements in bone scans, patient reported pain and analgesic use, circulating tumor cells, and bone biomarkers. The study was stopped because of these improvements in bone response. Patients treated with Cabozantinib showed a significant improvement in the primary end point of PFS compared with placebo group (median, 23.9 vs 5.9 weeks, respectively) [53]. Instead, in the following Phase III trial (COMET-1), Cabozantinib did not demonstrate a statistically significant increase in OS compared to prednisone. Indeed, COMET-1 showed a median OS of 11 months for treated patients and 9.8 months for the prednisone arm [54]. The most commonly reported adverse events with Cabozantinib included fatigue (13%), diarrhea (5%), and hypertension (5%) [54].

4.2. Targeting Cellular Src Kinase

The membrane-associated tyrosine kinase Src (encoded by the c-Src gene) is a proto-oncogene involved in the onset of several pathological processes such as tumor cell proliferation, adhesion, invasion, migration and metastasis development [55] [56].

One of the most studied Src inhibitor is dasatinib. In *in vivo* model of prostate cancer, it showed a synergistic effect when administered with docetaxel inhibiting the proliferation of prostate cancer cells implanted into bone [57].

A Phase II study of dasatinib in combination with docetaxel demonstrated safety and activity becoming the subject of the Phase III READY trial [58]. This study enrolled 1500 men with chemotherapy-naive mCRPC and randomized them to docetaxel and prednisone with or without dasatinib 100 mg daily. In this trial, mCRPC men naïve for chemotherapy and treated with dasatinib plus docetaxel did not show OS improvement. Indeed, the median OS in dasatinib and placebo group was 21.5 and 21.2 months respectively [59]. The most common grade 3 - 4 adverse events included diarrhea (8%) patients in the dasatinib group vs 4% patients in the placebo group,

fatigue (8% vs 6%), and asthenia (5% vs 3%) [59].

4.3. Targeting Endothelins

Endothelins (ET-1, ET-2, ET-3) are a group of 21-amino acid peptides that are produced in a variety of tissues, where they regulates the vasomotor tone, nociception, hormone production and cell proliferation [60]. It has been demonstrated that circulating levels of endothelin-1 (ET-1) in metastatic prostate cancer patients increased compared to patients with localized disease [60]. In the bone microenvironment, ET-1 alters osteoblasts/osteoclasts balance driving to new bone deposition that is typical of prostate cancer metastases [61]. Indeed, malignant cells release ET-1 that binds its receptor (Endothelin receptor A), expressed by osteoblasts, stimulating their proliferation and bone apposition activity. Activated osteoblasts in turn release several growth factors promoting survival and growth of bone metastatic cancer cells.

Zibotentan (ZD4054) is an oral, specific Endothelin receptor A antagonist under investigation in ENTHUSE clinical trials. In ENTHUSE M1 study in men with mildly symptomatic CRPC zibotentan treatment compared to placebo did not significant improve OS (24.5 vs 22.5 months, respectively) [62]. Moreover, the ENTHUSE M0 study evaluating zibotentan treatment in patients with non-metastatic CRPC has been discontinued because did not meet its primary end points (OS and progression-free survival [PFS]).

Finally, randomized Phase III ENTHUSE M1C trial investigating the effect of zibotentan in combination with docetaxel versus docetaxel plus placebo showed no improvements in OS, PSA response rate, time to PSA progression, PFS, time to new bone metastases, time to new SREs, pain response, or time to pain progression [63]. The most commonly reported adverse events in zibotentan-treated patients were peripheral edema (37.7%), headache (26.2%) and nasal congestion (24.9%); each occurred with >15% higher incidence than in the placebo group [63]. In view of these results, no further investigations with zibotetan are ongoing.

4.4. Immunotherapies

Prostate cancer represents an appealing setting for immunotherapy approaches given the relatively high expression of several tumor-associated antigens (TAAs) [64]. In this regard Sipuleucel-T has recently been approved in mCRPC and alternative strategies based on the possibility of interfering with the phenomenon of tumor immune escape are currently in development phases. The most promising approach appears to be the modulation of immunosuppressive micro-environment by acting on their co-inhibitory molecule [65]. Nowadays, CTLA-4 (cytotoxic T-lymphocyte associated antigen 4) is currently the most investigated, and the pharmacological approaches aimed at its inhibition are in advanced stages of clinical investigation also in metastatic prostate cancer [66] [67].

4.5. Sipuleucel-T

Sipuleucel-T is an autologous cellular immunotherapy indicated for the treatment of asymptomatic or minimally symptomatic metastatic CRPC. The patient's peripheral blood mononuclear cells are treated with a prostatic acid phosphatase-granulocyte macrophage colony-stimulating factor (PAP-GM-CSF) fusion protein in addition to various other cytokines to generate PAP-specific T cells capable of recognizing and killing prostate cancer cells that express PAP. This treatment was FDA-approved in 2010 for use in patients in mCRPC, based on the results of the pivotal Phase III trial (IMPACT). In this study 512 patients with asymptomatic or minimally symptomatic chemotherapy-naive mCRPC were randomized to receive sipuleucel-T versus placebo in a 2:1 ratio showing 4.1-month improvement in median OS (25.8 vs. 21.7 months) for sipuleucel-T compared with control (HR = 0.78; 95% CI: 0.61, 0.98) [68]. Common adverse reactions reported during a safety evaluation of 601 patients who received sipuleucel-T were chills, fatigue, fever, back pain, nausea, joint ache, and headache. The majority of adverse reactions were mild or moderate in severity [68].

4.6. Ipilimumab

Ipilimumab is a human monoclonal antibody that enhances and prolongs T-cell activation by blocking immune checkpoint CTLA-4 receptors found on the surface of T cells [65]. In a randomized Phase II trial, 108 patients with advanced prostate cancer treated with ipilimumab plus androgen-deprivation therapy showed undetectable PSA levels by 3 months compared with patients treated with endocrine therapy alone (55% vs 38%) [66].

Recently, the results from a randomized, double-blind Phase III study (CA-184-043) comparing ipilimumab with placebo following bone-directed radiation therapy in CRPC patients previously treated with docetaxel demonstrated no improvement in OS [67]. The most frequent grade 3-4 adverse events included diarrhea (16% in the ipilimumab group vs 2% in the placebo group), fatigue (11% vs 9%, anemia (10% vs 11%), and colitis (5% vs 0) [67].

Nevertheless, a subgroup analysis suggests that ipilimumab may be most active in patients with favorable laboratory prognostic factors (e.g., decreased alkaline phosphatase or elevated hemoglobin level) or in patients without visceral disease [66] (**Table 1**).

Table 1. Metastatic prostate cancer therapies currently approved or in clinical development.

Compound	Company	Structure	Stage of development	Mechanism of action
Targeting the androgen pathway				
Abiraterone	Janssen	Oral androgen biosynthesis inhibitor	Phase III	17 a-hydroxylase/C17,20-lyase (CYP17) inhibitor
Enzalutamide	Astellas	Oral hormonal therapy	Phase III	Androgen receptor antagonist
Orteronel	Takeda Pharmaceutical Company	Oral androgen biosynthesis inhibitor	Phase III	CYP17A1 inhibitor
Galeterone	Tokai Pharmaceuticals	Oral hormonal therapy and androgen biosynthesis inhibitor	Phase III	Both androgen receptor antagonist and an CYP17A1 inhibitor
ARN-509	Johnson & Johnson	Oral hormonal therapy	Phase III	Androgen receptor antagonist
ODM-201	Orion and Bayer Health Care	Oral hormonal therapy	Phase III	Androgen receptor antagonist
Targeting bone microenvironment				
Zoledronic Acid	Novartis	Intravenous bone resorption inhibitor	Phase IV	Farnesyl pyrophosphate sy50*ntetase inhibitor
Denosumab	Amgen	Subcoutaneus bone resorption inhibitor	Phase IV	RANK-L antibody
Radium-223	Bayer	Isotope of radium	Phase III	Radiopharmaceutical alpha-particles target
Targeting signal transduction pathways				
Cabozantinib	Exelixis	Oral small molecule TKi	Phase III	c-MET/HGF pathway inhibitor
Dasatinib	Bristol-Myers Squibb	Oral small molecule TKi	Phase III	c-Src/Abl kinase inhibitor
Zibotentan	AstraZeneca	Oral small molecule	Phase III	Endothelin receptor A antagonist
Immunotherapies				
Sipuleucel-T	Dendreon Corporation	Intravenous autologous cellular immunotherapy	Phase III	Immunomodulatory agent
Ipilimumab	Bristol-Myers Squibb	Intravenous monoclonal antibody	Phase III	CTLA-4 inhibitor and immunomodulatory agent
Other molecules				
Tasquinimod	Ipsen and Active Biotech	Oral small molecule tumor microenvironment inhibitor	Phase III	S100A9 inhibitor
Custirsen	Oncogenex Pharma	Intravenous antiapoptotic signalling inhibitor	Phase III	Clusterin production inhibitor

5. Other Molecules

5.1. Tasquinimod

Tasquinimod is a novel immunotherapy, orally active quinoline-3-caboxamide analog that targets the tumor microenvironment exerting immunomodulatory and antiangiogenic properties [69]. In particular, tasquinimod interferes with vascular tissue homeostasis downregulating the angiogenic suppressor thrombospondin-1 and upregulating Hypoxia-inducible factor 1-alpha (HIF-1 α). At the same times it is an inhibitor of S100A9 (a protein from the family of calcium-binding S100 proteins expressed on myeloid-derived suppressor cells) modulating the local tumor immunity. Indeed, it prevents the bind of S100A9 protein with its ligand inactivating proinflammatory cascade signaling pathways [70].

In a randomized phase II trial chemotherapy-naïve men with castrate-resistant prostate cancer showed a significant increase in progression free survival and overall survival with tasquinimod compared with placebo [71]. Tasquinimod was considered safe, with low to moderate side effects, which included mild gastrointestinal issues, muscle and joint pains, and fatigue [71]. Anyway, a phase III trial in 1245 patients did not confirm survival advantage. Even if there was a significant improvement in progression-free survival (median, 7.0 versus 4.4 months), there was no benefit in overall survival (median, 21.3 versus 24.0 months, 95%) [72].

5.2. Custirsen

Custirsen (OGX-011) is a second-generation, 2′-methoxyethyl-modified phosphorothioate antisense oligonucleotide that inhibits clusterin expression [73]. Clusterin is an antiapoptotic protein that preserves protein during cellular stress. In prostate cancer, clusterin overexpression is associated with a high Gleason score and has been detected in patients with mCRPC after neoadjuvant hormone therapy.

Studies of clusterin have demonstrated its antiapoptotic and prosurvival activities in prostate cancer that are believed to be associated with docetaxel resistance [74]. In a phase II trial custirsen (weekly intravenous administered) plus docetaxel extended median survival rates from 16.9 months to 23.8 months compared with single-agent docetaxel [75] [76], and a decrease of clusterin level after custirsen treatment was observed. In another Phase II trial custirsen was administered in combination with docetaxel or mitoxantrone as a second-line therapy in patients with mCRPC progressing after first-line docetaxel. Both combinations were well tolerated, but OS and PFS were better in the docetaxel arm (15.8 and 7.2 months vs 11.5 and 3.4 months, respectively) [76].

A randomized open-label phase III trial (SYNERGY) evaluated first-line therapy with custirsen in combination with docetaxel-prednisone versus docetaxel-prednisone alone in chemotherapy-naive mCRPC patients [77]. In particular, CRPC patients with a poor prognosis appeared to benefit from custirsen when added to docetaxel as 1st-line therapy. The poor prognosis group was analyzed separately for treatment effect (n = 492). The median OS was 17.0 m in the custirsen arm vs. 14.0 m in the control arm. PSA progression in the poor prognosis group also favored custirsen. Side effects included febrile neutropenia, fever, pleural effusion, and dyspnea [77].

Currently another randomized open-label phase III trial is ongoing in order to evaluate the OS, as first end point. In particular, AFFINITY trial, investigates the survival benefit in docetaxel-pretreated patients of second-line chemotherapy with cabazitaxel 25 mg/m^2 and prednisone 10 mg/day with or without custirsen [78].

6. Conclusions

The treatment of prostate cancer, in particular of its most malignant hormone independent and castration resistant forms, has been improved thanks to the develop of new strategies.

In the last few years, many different therapeutic strategies for CRPC have been developed and evaluated in clinical studies. Several strategies showed objective clinical benefit and have been approved for clinical use. These include the androgen inhibitors such as enzalutamide and abiraterone, radium-223 and sipuleucel-T. Although enzalutamide, abiraterone and radium-223 represent the standard care for the treatment of bone metastatic CRPC, sipuleucel-T has not widely used in clinical practice because, rarely, patients responded to therapy. The identification of biomarkers could help to select patients that may (or may not) benefit from sipuleucel-T therapy.

In the near future, it will be crucial to test these agents together or sequentially. In this regard a Phase II study on CRPC bone metastatic patients in which abiraterone and enzalutamide were administered simultaneously showed promising results [73]. Moreover, recent evidence has demonstrated that anti-androgens are able to tar-

get both prostate cancer cells and the bone microenvironment. This could influence future therapeutic approaches evaluating the possibility of combining anti-androgen treatment with bone-targeted agents (biphosphonates, denosumab) in order to achieve better control of prostate cancer bone metastases. In recent years, the simultaneous development of novel and more potent classes of drugs targeting androgen pathway has emerged such as ortonel, ARN-509 and galeterone.

On the other hand, there are several examples of new molecular targeting drugs that showed promising results in preclinical PCa models but showed insufficient effects in clinical phase III studies. These targets include the endothelin A receptor antagonist zibotentan, the Src inhibitor dasatinib, and the c-MET inhibitor cabozantinib, although some of the initial excitement failed to materialize.

In spite of the development and consequent approval of a number of new drugs active against mCRPC, no great improvement in overall was found and several patients have disease progression and early mortality. The discovery of new drug resistance mechanisms, the genomic and proteomic analysis to classify different prostate cancer molecular subtypes, as well as the set-up of new prognostic and predictive biomarkers, may further improve mCRPC treatment.

In order to make a great deal of progress in prostate cancer treatment, it is imperative that both basic and clinical investigators cooperate to reach this common goal.

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Abbreviations

PCa: Prostate Cancer
PIN: Prostatic Intraepithelial Neoplasia
HGPIN: High Grade Prostatic Intraepithelial Neoplasia
ADT: Androgen Deprivation Therapies
PSA: Prostate Specific Antigen
SRE: Skeletal Related Event
mCRPC: metastatic Castration Resistant Prostate Cancer
OS: Overall Survival
AR: Androgen Receptor
CYP17: Cytocrome P450 17 alpha hydroxysteroid dehydrogenase
PFS: Progression Free Survival
RANK: Receptor Activator of Nuclear Factor-Kb
RANKL: Receptor Activator of Nuclear Factor-kB Ligand
OPG: Osteoprotegerin
TNF: Tumor Necrosis Factor
TGF-b: Tumor Growth Factor-b Factor
PDGF: Platelet-Derived Growth Factor
bALP: bone alkaline phosphatase
uNTX: urine N-telopeptide
HGF: Hepatocyte Growth Factor
VEGF: Vascular Endothelial Growth Factor
VEGFR: Vascular Endothelial Growth Factor Receptor
ET: Endothelin
TAAs: Tumor Associated Antigens
CTLA-4: Cytotoxic T-lymphocyte associated antigen 4
PAP-GM-CSF: Phosphatase-granulocyte macrophage colony-stimulating factor
HIF-1α: Hypoxia-Inducible Factor 1-alpha