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New Drugs

Emerging therapeutic targets for patients with advanced prostate cancer

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ABSTRACT

Although recent advances in the treatment of castration-resistant prostate cancer (CRPC) have significantly improved patient outcomes, advanced prostate cancer is still associated with substantial morbidity and mortality, particularly in patients who develop resistance after multiple lines of therapy. Various cell signaling, DNA repair, and epigenetic enzymatic pathways are being targeted with small-molecule inhibitors in order to identify treatment strategies for patients with CRPC. In this review, we discuss novel targets and agents, studied preclinically and now being validated in clinical trials, including poly ADP-ribose polymerase (PARP), enhancer of zeste homologue 2 (EZH2), hedgehog pathway, MDM2/p53, and tyrosine kinase inhibitors. Further, we outline current approaches for novel prostate cancer vaccines such as DCVAC/PCa, PROSTVAC-V/F, MVI-816, CV9104, and PF-06753512. This wide spectrum of potential treatment strategies holds promise for additional improvements in the treatment of patients with CRPC, as these novel agents are aimed at targets known to be associated with growth and malignant progression of prostate cancer. If primary study endpoints are met, findings from ongoing phase III trials of well-tolerated and active combinations may provide new effective treatment options for advanced prostate cancer and thereby contribute to enhanced disease control in CRPC patients.

Introduction

Prostate cancer represents the leading cause of cancer in men, with 164,690 new cases expected to be diagnosed in the United States, in 2018 [1]. Significant progress has been made in the past several years in the treatment of castration-resistant prostate cancer (CRPC), with the approval of effective agents with unique mechanisms of action, including docetaxel, enzalutamide, abiraterone acetate, cabazitaxel, sipuleucel T, radium 223 dichloride, and apalutamide [2–10]. Nonetheless, clinically significant prostate cancer remains associated with substantial mortality, as it currently represents the second most common cause of cancer death in men (9% of all cancer deaths), due to the development of acquired resistance to sequential lines of treatment and the associated disease progression [1,11,12]. Particularly challenging is the treatment of patients with metastatic CRPC (mCRPC), who have, on average, a 5-year survival rate of only ~15–36% [1,12].

The discovery and current use of novel immunotherapeutic approaches have substantially improved treatment outcomes in a number of cancer settings (e.g., advanced melanoma, renal cell carcinoma, head and neck cancer, and lung cancer). However, in patients with prostate cancer, efficacy of single-agent, immunotherapeutic agents such as programmed cell death 1 (PD-1), PD-ligand 1 (PD-L1), and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) antagonists appears to be limited to a small proportion of patients [13,14]. Evaluation of novel combinations with other agents (e.g. small-molecule inhibitors [SMIs], vaccines, or other immunomodulating agents), currently in progress in multiple clinical studies, may ultimately yield treatment regimens associated with improved outcomes.

Thus, the epidemiological impact of advanced prostate cancer and current patient outcomes still underscore the need to identify more effective treatment strategies for CRPC [1,11,12]. Since CRPC patients are oftentimes men older than 70–75 years of age with associated comorbidities, new treatment options should have well-tolerated safety profiles [15].

The pace and breadth of preclinical discovery as well as the clinical development of new agents have recently accelerated in the prostate cancer setting, with a substantial number of new agents comprising different mechanisms of action and novel structures/composition. In

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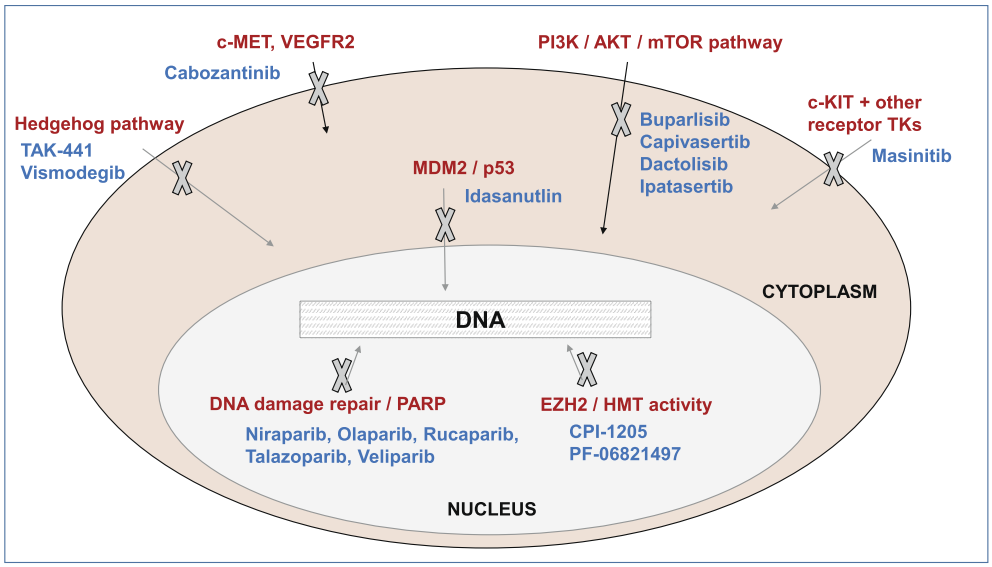

Fig. 1. Signaling, DNA repair, and epigenetic pathways targeted by small-molecule inhibitors currently being investigated in patients with advanced prostate cancer. EZH2: enhancer of zeste homologue 2; HMT: histone methyl-transferase; MDM2: mouse double minute 2 homolog; MET: mesenchymal-epithelial transition factor; mTOR: mammalian target of rapamycin; PARP: poly-ADP ribose polymerase; PI3K: phosphatidylinositol-4,5-bisphosphate 3-kinase; TK: tyrosine kinase; VEGFR2: vascular endothelial growth factor receptor 2.

this review, we will discuss novel, non-hormonal targets that are emerging for the treatment of patients with advanced prostate cancer, including signaling and epigenetic pathways inhibited by SMIs, and the latest developments in novel therapeutic vaccines.

Small-molecule inhibitors of signaling, DNA repair, or epigenetic pathways

Poly ADP-ribose polymerase (PARP) inhibition

Inhibition of PARP is currently one of the most active fields of investigation in the development of new agents for patients with CRPC, as the PARP enzyme plays a key role in the repair of DNA damage induced by multiple causes (e.g. UV light, radiation, chemotherapy) [16–19] (Fig. 1). Both PARP1 and PARP2 mediate repair of single-stranded (ss) DNA breaks by base-excision repair. If PARP is unable to repair ssDNA breaks due to the presence of an inhibitor, proteins of the homologous recombination repair (e.g. BRCA1, BRCA2) can compensate by repairing double-stranded (ds) DNA breaks. In patients with germline or somatic defects in these DNA repair genes (> 20% in mCRPC), PARP inhibition effectively blocks DNA repair in the target cancer cell leading to cell death [17]. In preclinical studies, inhibition of PARP1 by small-interfering RNA was shown to inhibit tumor growth, invasiveness, and disease progression in xenograft tumor models of prostate cancer [18].

Three PARP inhibitors, olaparib, rucaparib, and talazoparib, are being evaluated in phase III trials in mCRPC and results are eagerly awaited (Table 1). Findings from prior phase I/II studies indicate that PARP inhibitors have an established and relatively well-tolerated safety profile and may induce responses in a substantial proportion of patients with DNA-repair defective (DRD+) tumors. Treatment with PARP inhibitors may be associated with the development of grade 3–4 anemia, thrombocytopenia, neutropenia, and hypertension, which require monitoring and treatment interruption or discontinuation in a few cases [16,20–30].

Combination of DNA repair inhibitors with DNA damage-inducing agents, such as chemotherapy, radiation therapy, and other cytotoxic agents, may enhance the effectiveness of PARP inhibitors. Thus, investigators are working to identify the most effective and tolerable combination modalities and sequencing strategies for the use of PARP inhibitors in patients with mCRPC. Ongoing studies are also assessing which genetic abnormalities/biomarkers (i.e. defects in homologous recombination DNA repair genes such as *BRCA1/2, ATM, CHEK1, CHEK2, WES, BARD1, BRIP1, FAM175A, MRE11A, NBN, PALB2, RAD51C,* and *RAD51D*) would be most useful for selecting patients with tumors sensitive to PARP inhibition, as reviewed by Stover et al. [31].

Olaparib inhibits the catalytic activities of both PARP1 and PARP2 and it is approved for previously treated patients with deleterious or suspected deleterious germline *BRCA*-mutated metastatic HER-negative breast cancer and ovarian cancer. In prostate cancer, *in vitro* exposure of wild-type p53 LNCaP cells to olaparib resulted in proliferative arrest and apoptosis within a week. LNCaP cells harbor multiple mutations in dsDNA repair pathway genes, including *BRCA2, RAD51B, RAD54L, ATM,* and *FANCA,* suggesting that one or more genetic abnormalities may confer sensitivity to PARP inhibition [20]. In the clinic, high response rates (RRs) were observed in a phase II study of olaparib conducted in mCRPC patients with defects in DNA-repair genes, who had received multiple lines of prior treatment [21]. Anemia (20%) and fatigue (12%) were the most frequently reported grade 3–4 adverse events (AEs). Confirmed, radiologic partial responses were observed in 19% of evaluable patients [21].

Olaparib is currently being evaluated in a multicenter, randomized, open-label phase III trial (PROfound, NCT02987543) versus abiraterone or enzalutamide in patients who have failed prior hormonal therapy and have somatic or germline mutations in homologous recombination repair genes (primary endpoint: radiographic PFS [rPFS]). Results from a randomized phase II study of olaparib in combination with abiraterone versus abiraterone in patients previously treated with docetaxel demonstrated a significant improvement in median rPFS with the combination (13.8 v 8.2 months; hazard ratio 0.65; $p = 0.034$). Such improvement appeared to be maintained regardless of DRD status. More patients experienced grade ≥ 3 AEs (i.e. anemia, pneumonia, and myocardial infarction) with the combination than with abiraterone alone (54% v 28%) [22]. A phase III study is being planned.

The increased mutational burden resulting from PARP inhibition, which leads to cancer cell damage and potential release of tumor-associated antigens (TAAs), may provide a new strategy to facilitate antitumor immune responses elicited by anti-PD-1 or anti-PD-L1 antibodies [13,23]. Thus, additional phase I/II studies in mCRPC are assessing safety and activity of olaparib in combination with the anti-PD-1 antibody pembrolizumab or the anti-PD-L1 antibody durvalumab. The combination of olaparib and pembrolizumab will be compared with pembrolizumab plus docetaxel/prednisone, pembrolizumab plus enzalutamide, and pembrolizumab plus abiraterone/prednisone (NCT02861573). In addition, olaparib is being evaluated in combination with radium 223 dichloride in patients with mCRPC and bone metastases (NCT03317392).

Rucaparib, approved both for the treatment of deleterious BRCA mutation-associated epithelial ovarian, fallopian tube, or primary

Table 1
Small-molecule inhibitors in clinical development for the treatment of patients with advanced prostate cancer.

Target and mechanism of action	Agent	Company	Patient population	Phase
EZH2 (HMT) inhibitors	CPI-1205	Constellation Pharmaceutical	mCRPC	I-II
	DS-3201b	Daiichi Sankyo	leukemia, lymphoma	I
	PF-06821497	Pfizer	mCRPC	I
	Tazemetostat (EPZ-6438)	Epizyme	sarcoma, lymphoma	I-II
Hedgehog inhibitors	TAK-441	Takeda	mCRPC	I
	Vismodegib	Roche	mCRPC	I
MDM2 inhibitor	Idasanutlin (RG7388)	Roche	mCRPC	I
PARP inhibitors	Niraparib	Tesaro	DRD+ mCRPC	II
	Olaparib	AZ/Merck	DRD+ mCRPC	III
	Rucaparib (CO-338)	Clovis Oncology (Pfizer)	DRD+ mCRPC	III
	Talazoparib (MDV 3800/BMN 673)	Pfizer/Medivation	DRD+ mCRPC	III
	Veliparib (ABT-888)	Abbott	DRD+ mCRPC	II
PI3K/AKT/mTOR inhibitors	Buparlisib (BKM120)	Novartis	mCRPC	II
	Capivasertib (AZD5363)	Astra Zeneca	mCRPC	II
	Dactolisib (BEZ235)	Novartis	mCRPC	II
	Ipatasertib (GDC-0068, RG7440)	Roche	mCRPC	III
Tyrosine kinase inhibitor c-KIT R	Masitinib (AB1010)	AB Science	mCRPC	III
Tyrosine kinase inhibitor (c-MET, VEGFR2)	Cabozantinib (XL-184)	Exelixis	mCRPC	III

c-KIT R: c-KIT receptor; DRD+: with DNA repair defects; EZH2: enhancer of zeste homologue 2; HMT: histone methyl-transferase; mCRPC: metastatic castration-resistant prostate cancer; MDM2: mouse double minute 2 homolog; MET: mesenchymal-epithelial transition factor; mTOR: mammalian target of rapamycin; PARP: poly-ADP ribose polymerase; PI3K: phosphatidylinositol-4,5-bisphosphate 3-kinase; PTEN: phosphatase and tensin homolog; VEGFR2: vascular endothelial growth factor receptor 2.

peritoneal cancer and for maintenance therapy of these cancers, is being evaluated in a multicenter, randomized, open-label phase III study (TRITON3, NCT02975934) versus physician's choice of therapy in patients with mCRPC associated with homologous recombination deficiency (i.e., deleterious mutations in *BRCA1/2* or *ATM* genes) [24]. The primary endpoint of the study is rPFS.

Talazoparib is a PARP inhibitor that inhibits both PARP1 and PARP2 and traps PARP on the DNA strand, thus preventing DNA damage repair. It has an enzymatic inhibitory activity comparable to that of olaparib and rucaparib, but it is more effective in trapping PARP–DNA at the site of DNA damage. It is also ~100-fold more cytotoxic than olaparib and rucaparib in combination with DNA alkylating agents [25]. In a phase I trial conducted in patients with *BRCA1/2* mutation–associated cancers, talazoparib demonstrated a well-tolerated safety profile and antitumor activity as monotherapy, with a 50% and 42% RR in advanced breast and ovarian cancer, respectively. Objective responses were also observed in patients with other solid tumors [26].

A randomized, phase III trial (TALAPRO-2, NCT03395197) is evaluating talazoparib in combination with enzalutamide versus placebo plus enzalutamide in patients with DRD+ mCRPC, with a primary endpoint of rPFS [27]. A phase II study is assessing response to talazoparib in patients with DRD+ mCRPC, who have previously received taxane-based chemotherapy and progressed on at least one hormonal agent (enzalutamide and/or abiraterone/prednisone). In addition, CRPC patients with *BRCA* or *ATM* gene defects are being enrolled in a phase II study (Javlin PARP Medley, NCT03330405) for investigational treatment with talazoparib in combination with the PD-L1 inhibitor avelumab.

The PARP inhibitor veliparib was shown in an early study to induce a response in a small number of *BRCA2*-mutated mCRPC patients, one of whom had systemic disease involving liver and bone [28]. Further evaluation of veliparib in a phase II study (NCI 9012) randomizing mCRPC patients to veliparib plus abiraterone/prednisone versus abiraterone/prednisone showed no significant difference in PSA RR and mPFS between the two study arms [29]. The patient ETS fusion status did not correlate with response to treatment. However, DRD+ patients in both treatment groups had a significantly higher PSA RR and longer mPFS (14.5 v 8.1 months) compared with patients with wild-type tumors, suggesting potential interactions between androgen signaling and DNA repair activity. Fatigue, lymphopenia, nausea, and vomiting were observed more frequently with combination treatment. Grade 3 treatment-related AEs were reported in 24% of patients with the combination and 20% of patients with abiraterone alone [29].

The PARP1 and PARP2 inhibitor niraparib, which is approved for the maintenance treatment of patients with platinum-sensitive, recurrent ovarian cancer, has demonstrated improved PFS versus placebo in this population, although benefit was greater in patients with germline *BRCA1/2* mutations and homologous recombination deficiency [30]. The most frequent, treatment-related, grade 3–4 AEs reported in this phase III trial were thrombocytopenia (34%), anemia (25%), and neutropenia (20%) [30]. Niraparib is currently being assessed as monotherapy in an open-label phase II study (Galahad, NCT02854436) in patients with DRD+ mCRPC and combined with the anti-PD-1 antibody JNJ-63723283 in a phase I/II study in patients with mCRPC (NCT03431350). Furthermore, following a phase I study combining niraparib with abiraterone/prednisone, a phase III trial will evaluate niraparib plus abiraterone/prednisone versus abiraterone/prednisone in mCRPC (NCT03748641).

Enhancer of zeste homologue 2 (EZH2) inhibition

EZH2 is a histone methyltransferase (HMT), the enzymatic subunit of the Polycomb repressive complex-2 (PRC2), which induces transcriptional silencing of target genes by methylation of lysine 27 residues on histone H3 (H3K27 methylation) 32]. Abnormalities in EZH2 have been linked to cancer development and progression. EZH2 gain-of-function mutations resulting in elevated levels of H3K27me3 have been identified in follicular lymphoma and diffuse large B-cell lymphoma (DLBCL) and found associated with disease progression [32–34]. In addition, EZH2 overexpression correlated with disease progression and poorer prognosis in several solid tumors including prostate, breast, bladder, endometrial, and small-cell lung cancer (SCLC) [35,36]. Increased EZH2 activity may lead to suppression of cancer cell differentiation and tumor-suppressor pathways [37].

In prostate cancer, EZH2 was shown to promote tumor growth in preclinical models, with EZH2 inhibition resulting in decreased proliferation of prostate cancer cells *in vitro* and *in* vivo, and increased antitumor activity of androgen-receptor (AR) antagonists in CRPC [35–40]. In addition, recent studies highlighted the potential for EZH2 inhibition to overcome CRPC resistance to the AR antagonist enzalutamide, by inducing expression of genes which inhibit AR signaling

and are co-repressed by AR and EZH2 (e.g. *CCN3*) [41,42]. EZH2 inhibition was also shown to prevent cellular reprogramming and differentiation of prostate tumors into enzalutamide-resistant neuroendocrine prostate cancer (NEPC) [43–45].

Based on the increasing evidence for the role of EZH2 in cancer progression, novel EZH2 inhibitors are being evaluated in patients with mCRPC and other advanced malignancies. These EZH2 inhibitors include CPI-1205, PF-06821497, tazemetostat, and DS-3201b. CPI-1205 is a selective and cofactor-competitive EZH2 inhibitor that blocks the catalytic activity of wild-type and mutant EZH2, which demonstrated antitumor activity in patients with non-Hodgkin lymphoma (NHL) [46,47]. In this population, the most common treatment-related AEs were anemia, nausea, diarrhea, and fatigue; lymphopenia was the grade ≥ 3 AE reported in > 1 patient (9%) [46]. A randomized phase Ib/II study (ProSTAR, NCT03480646) is evaluating CPI-1205 with enzalutamide or abiraterone/prednisone in patients with mCRPC after prior AR-inhibitor therapy and a phase I/II study (ORIOn-E, NCT03525795) is exploring a combination of CPI-1205 with the anti-CTLA-4 antibody ipilimumab in patients with advanced solid tumors [48]. Based on preclinical findings, EZH2 inhibitors may contribute to improved outcomes in combination with antiandrogen therapies in both early- and late-stage mCRPC patients, through increased antitumor activity and potential re-sensitization of tumors resistant to hormone therapy.

PF-06821497 is a selective, potent EZH2 inhibitor with an inhibitory constant of < 0.1 nM against both wild-type and mutant Y641N-EZH2, with no significant activity against other HMTs. *In vivo*, PF-06821497 induced dose-dependent tumor growth inhibition in DLBCL xenograft models, in correlation with its pharmacodynamic inhibition of H3K27me3 methylation in tumor tissues [49]. A multicenter, phase I study (NCT03460977) is evaluating safety and antitumor activity of PF-06821497 in patients with CRPC (in combination with standard of care), SCLC (in combination with cisplatin or carboplatin plus etoposide), and NHL. The EZH2 inhibitor tazemetostat is also being assessed in patients with sarcomas, NHL, and other advanced malignancies [50]. DS-3201b is a dual inhibitor, targeting both EZH1 and EZH2, which is being tested in phase I studies in patients with lymphoma and leukemia [51]. EZH2 inhibitors currently in clinical trials have shown well-tolerated safety profiles with promising responses in NHL. The trials described above will help delineate early efficacy signals for EZH2 inhibitors in mCRPC.

Hedgehog (Hh) pathway inhibition

Increased activity of the Hh pathway, which is physiologically involved in organ development and regulation of stem cells through regulation of GLI-mediated transcription, has been reported in many tumor types. In prostate cancer, abnormal Hh pathway signaling may increase neoplastic transformation and epithelial-to-mesenchymal transition, leading to tumor dissemination. Further, it may contribute to a castration-resistant phenotype, due to cross-talk between the Hh and the AR pathways, and other effects on the tumor microenvironment [52].

Two Hh pathway inhibitors have been evaluated in mCRPC, TAK-441, an investigational agent, and vismodegib, approved for the treatment of patients with advanced basal cell carcinoma (BCC) [53–55]. In preclinical tumor models, progression of castration-resistant LNCaP xenografts was significantly delayed following exposure to the Hh/Smoothened inhibitor TAK-441. Expression of murine Hh-activated genes (*Gli1*, *Gli2,* and *Ptch1*), was also reduced, suggesting that TAK-441 may delay tumor progression in CRPC by disrupting Hh pathway-mediated paracrine signaling in the tumor microenvironment [53]. In a phase I trial, *GLI1* expression was strongly reduced in skin biopsies from patients with advanced solid tumors, although only a patient with BCC had a partial response [54]. The effects of treatment with vismodegib in mCRPC were evaluated in a pharmacodynamic study, with determination of *GLI1* expression levels in soft-tissue metastases. *GLI1* mRNA expression levels showed a > 50% decrease in post-treatment versus pre-treatment biopsies in ~57% of patients. However, none of the patients had prostate-specific antigen (PSA) or measurable tumor responses [55].

Findings in an earlier disease setting, from a randomized, neoadjuvant study of the Hh pathway inhibitor sonidegib (LDE-225) versus observation, in patients with high-risk localized prostate cancer undergoing prostatectomy, showed a > 60-fold decrease in *GLI1* expression and Hh pathway inhibition. Treatment was well tolerated, but mPFS was comparable between study arms, suggesting that further investigations are needed to identify a potential benefit from Hh pathway inhibition in prostate cancer [56]. Further studies may also clarify whether new strategies aimed at inhibiting SMO-independent GLI activation, mediated by direct binding of AR to GLI proteins in prostate cancer cells, may translate into an effective therapeutic approach for CRPC [57].

MDM2 inhibition

The protein MDM2 (an E3 ubiquitin-protein ligase) is a negative regulator of p53, which is a potent tumor suppressor able to induce cell cycle arrest and death following activation by cellular or oncogenic stress [58]. As the majority of prostate cancers have functional p53, inhibition of MDM2 by specific antagonists (i.e. the *cis*-imidazoline analogs Nutlins) may release activity of endogenous p53, thus resulting in cell cycle arrest and apoptosis in target cancer cells. Furthermore, due to signaling pathway cross-talk between p53 and AR, activation of p53 may increase the antitumor effects of androgen deprivation in prostate cancer [59–61]. Consistent with this hypothesis, combination treatment with Nutlin-3a and androgen deprivation in a LNCaP xenograft animal model resulted in tumor regression and a substantial increase in survival [59].

In patients with mCRPC, preliminary pharmacodynamic results from a dose-finding study of the second-generation MDM2 antagonist idasanutlin in combination with abiraterone/prednisolone or enzalutamide showed p53 activation and induction of macrophage inhibitory cytokine-1 [62,63]. A phase I trial (NCT03362723) is evaluating various oral formulations of idasanutlin in patients with advanced solid tumors.

Phosphatidylinositol-4,5-bisphosphate 3-kinase/AKT/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway inhibition

The PI3K/AKT/mTOR signaling pathway is known to regulate growth, proliferation and survival of cancer cells. Within this pathway, the tyrosine kinase (TK) AKT plays a central role in mediating its oncogenic effects. Multiple mechanisms may lead to activation of AKT signaling in tumor cells, including activating mutations in AKT or in the p110α subunit of PI3K (PIK3CA), loss of the tumor suppressor phosphatase and tensin homolog (PTEN), and/or increased receptor signaling [64]. Multiple PI3K/mTOR signaling inhibitors have thus been investigated to assess their therapeutic potential in various solid and hematologic malignancies (e.g. advanced breast, colorectal, ovarian, endometrial, urothelial, and NSCLC), as well as prostate cancer [64].

In CRPC, combination treatment with the pan-PI3K inhibitor buparlisib or the dual pan-PI3K/mTOR inhibitor dactolisib and abiraterone acetate/prednisone in a phase Ib study was associated with PSA responses in a few patients but no objective responses. More patients experienced dose-limiting toxicities with dactolisib than with buparlisib, as expected [65]. Similar findings were reported from a phase II study assessing buparlisib (with or without enzalutamide) in patients with mCRPC and disease progression after multiple lines of prior treatment. PSA declines were observed in 23% of patients, with no radiographic responses [66]. Common grade 1–2 AEs were diarrhea, nausea, weight loss, fatigue, anorexia, rash, hyperglycemia, and

anxiety/mood disorders. Grade 3 AEs were reported in 43% of patients. Serious AEs affecting 4 (13%) patients included respiratory infection and multi-organ failure, urinary tract obstruction, confusion, and seizure. Dose optimization and selection of patients most likely to respond (such as those with PTEN loss) may contribute to improve tolerability and activity of this combination treatment [66].

Novel, more selectively targeted inhibitors are currently being developed to improve on the safety profile and tolerability of agents designed to inhibit PI3K/AKT/mTOR signaling. Ipatasertib is a selective, ATP-competitive inhibitor of all 3 AKT isoforms [67]. In a phase I study, treatment in patients with advanced solid malignancies showed a tolerable safety profile, downregulation of multiple AKT effectors (including pPRAS40, pGSK3β, pS6, and p4EBP), and achievement of stable disease in ~44% of patients treated at the maximum tolerated dose of ipatasertib [68]. Radiographic evidence of stable disease as best response was observed in ~67% of patients who had PTEN loss or mutations in *PIK3CA* or *AKT* in their tumors. A patient with CRPC had a sustained prolongation of PSA doubling time; none of the patients had a partial or complete response by RECIST. A randomized, phase III trial (IPATential150, NCT03072238) is testing ipatasertib combined with abiraterone plus prednisone in patients with asymptomatic/mildly symptomatic, previously untreated mCRPC.

Similarly, the SMI capivasertib inhibits all AKT isoforms and its activity appears linked to the presence of *PIK3CA* mutations and/or PTEN loss in target cells. Conversely, the presence of *RAS* mutations may confer resistance to capivasertib [69]. In preclinical studies, capivasertib inhibited tumor growth in xenografts models and showed enhanced antitumor activity in combination with docetaxel or enzalutamide [70]. Based on these preclinical findings, a phase I/randomized phase II trial (ProCAID, NCT02121639) of capivasertib in combination with docetaxel/prednisone is in progress in patients with mCRPC, as well as a randomized phase II study (RE-AKT, NCT02525068) in combination with enzalutamide (versus placebo plus enzalutamide) in patients with mCRPC who have received prior chemotherapy and abiraterone. In addition, a phase I study (NCT03310541) is investigating capivasertib in combination with enzalutamide in previously treated, prostate cancer patients with specific *AKT* mutations.

Inhibition of other TK pathways

Masitinib targets wild-type and mutated c-KIT and other receptor TKs, including fibroblast growth factor receptor-3, platelet-derived growth factor receptor (PDGFR) α/β, lymphocyte-specific protein TK (Lck), Lck/Yes-related novel protein TK, and focal adhesion kinase [71]. It can thus influence survival and differentiation of macrophages and mast cells, which may be involved in angiogenesis, tissue remodeling, and immunomodulation. Findings from a phase II study in second-line mCRPC showed an acceptable safety profile and a longer overall survival (OS) with masitinib in combination with docetaxel (18.4 months) compared with historical results (13.8 months) prior to the introduction of enzalutamide therapy [71]. A multicenter, randomized, double-blind, phase III study (AB12003) is in progress to compare the efficacy and safety of masitinib in combination with docetaxel/prednisone versus docetaxel/prednisone in first-line mCRPC. The study will assess PFS as the primary endpoint and OS as the key secondary endpoint.

Cabozantinib/XL-184, a c-MET/vascular endothelial factor receptor 2 (VEGFR2) inhibitor that can also inhibit the AXL and RET TK receptors, is approved for the treatment of patients with advanced renal cell carcinoma [72]. Results from a large, randomized, discontinuation, phase II study demonstrated a disease control rate > 40% in different tumor types and a significant improvement in PFS with cabozantinib in patients with CRPC versus placebo, which led to the early suspension of randomization to placebo due to ethical considerations and continuation with open-label expansion cohorts for CRPC and ovarian cancer patients. The most frequent grade 3–4 AEs were fatigue, hypertension, and palmar-plantar erythrodysesthesia syndrome (< 16% of patients) [72]. However, results from a randomized, phase III trial (COMET-1) showed that although rPFS was significantly prolonged with cabozantinib versus prednisone (median, 5.6 vs 2.8 months; hazard ratio, 0.48), there was no significant improvement in OS (primary study endpoint) in post-chemotherapy patients with mCRPC [73].

Agents with anti-angiogenic and multi-kinase inhibitory activity such as cabozantinib may act synergistically with immunomodulatory agents (i.e. anti-PD-1/PD-L1 antibodies), by inducing vascular normalization in tumors and allowing more effective infiltration of immune effectors in tumors, and by neutralizing myeloid-derived suppressor cells [74]. Thus, following initial evaluation of cabozantinib in combination with immune checkpoint inhibitors in patients with renal cell carcinoma and other genitourinary tumors, an open-label, phase I-II study (NCT03170960) is currently assessing combined treatment with cabozantinib and the PD-L1 antagonist atezolizumab in patients with mCRPC and other malignancies.

In conclusion, multiple signaling, DNA repair, or epigenetic pathways are being targeted with SMIs to identify better treatment strategies for patients with CRPC. As recently confirmed by whole-genome sequencing analysis of tissue samples obtained from patients with advanced prostate cancer, human tumors may be quite heterogeneous within a patient, expressing different molecular profiles in different metastases (e.g., disease-involved lymph nodes or bone lesions) [75]. Nonetheless, a better understanding of the key genetic/biologic factors conferring sensitivity to treatment may lead to the identification and validation of biomarkers suitable for selecting the patients most likely to respond to specific agents.

Current clinical findings indicate that PARP inhibitors may contribute to improved outcomes in patients with mCRPC. Results from the ongoing phase III trials of PARP inhibitors combined with antiandrogen agents, in first line or following development of resistance to hormone therapy and/or chemotherapy, will contribute to define their safety/tolerability and potential therapeutic benefit in these settings. Owing to their immunomostimulatory activity, combinations with immune checkpoint inhibitors (i.e. anti-PD-1/PD-L1 antibodies) might increase the use of PARP inhibitors in early lines of CRPC treatment, if found suitably safe and effective. Furthermore, phase III clinical trial results will clarify whether the kinase inhibitors ipatasertib, capivasertib, and masinitib may represent effective and well tolerated therapeutic options for first-line or later treatment of patients with mCRPC, in combination with hormone therapy or chemotherapy.

Emerging targets: vaccine-based immunotherapy

The overexpression of TAAs, such as PSA, prostate-specific membrane antigen (PSMA), and prostatic acid phosphatase (PAP) by prostate cancer cells, has provided the rationale for development of specific immunotherapeutic and vaccination approaches for patients with CRPC [76]. Although extensive preclinical research and multiple attempts have been made in the past, the first immunotherapy, sipuleucel-T, was approved in prostate cancer in 2010. Sipuleucel-T is a cell-based, autologous therapy indicated for patients with asymptomatic/minimally symptomatic mCRPC. It requires a fairly complex process with collection of peripheral blood mononuclear cells (PBMCs) by leukapheresis and *ex-vivo* activation with a recombinant fusion protein (PA2024) consisting of PAP fused to granulocyte-macrophage colony stimulating factor (GM-CSF), prior to reinfusion [8]. Sipuleucel-T demonstrated a favorable safety profile and a statistically significant improvement (~4.1 months) in median OS versus placebo in the pivotal phase III trial, although it did not significantly prolong time to disease progression [8].

Table 2
Novel vaccine-based immunotherapeutic approaches for patients with prostate cancer.

Vaccine	Composition and Targets	Company	Phase of Development
CV301 (CEA-MUC-1-TRICOM)	Poxviral-based (modified vaccinia and fowlpox), CEA- and MUC1-targeted vaccine with multiple T-cell costimulatory molecules (B7.1, ICAM-1, and LFA-3) (CEA-MUC-1-TRICOM)	Bavarian Nordic/ National Cancer Institute	I–II
CV9103	mRNA vaccine (PSA, PSCA, PSMA, STEAP1)	CureVac	I–II
CV9104	mRNA vaccine (PSA, PSCA, PSMA, STEAP1, PAP, MUC1)	CureVac	IIb
DCVAC/PCa	Autologous dendritic-cell vaccine	SOTIO	II–III
MVI-816 (pTGV-HP)	Plasmid DNA vaccine (encoding PAP) + adjuvant GM-CSF + anti-PD-1	Madison Vaccines	I
PF-06753512 (PrCa VBIR1)	Vaccine-based immunotherapy regimen (adenovirus priming + DNA boost vaccination [PSA, PSMA, PSCA] + local CTLA4 antagonist + sunitinib or anti-PD-1 mAb)	Pfizer	I
PROSTVAC-VF (rilimogene) (PSA-TRICOM)	Poxviral-based (vaccinia and fowlpox), PSA-targeted vaccine with multiple T-cell costimulatory molecules (B7.1, ICAM-1, and LFA-3)	Bavarian Nordic	III (terminated study)

ADT: androgen-deprivation therapy; CEA: carcinoembrionic antigen; CTLA-4: cytotoxic T lymphocyte-associated antigen; GM-CSF: granulocyte-macrophage colony-stimulating factor; mAb: monoclonal antibody; MUC1: mucin 1; PAP: prostatic acid phosphatase; PD-1: programmed cell death 1; PrCa VBIR: vaccine-based immunotherapy regimen for prostate cancer; PSA: prostate-specific antigen; PSCA: prostate stem-cell antigen; PSMA: prostate-specific membrane antigen; STEAP1: six-transmembrane epithelial antigen of prostate 1.

DCVAC/PCa

DCVAC/PCa consists of an active, cellular immunotherapy with an autologous dendritic cell (DC) vaccine (Table 2). Autologous DCs are collected from the peripheral blood of each patient by leukapheresis, followed by gradient-based separation of PBMCs and culture of adherent cells. Immature DCs are activated *in vitro* with polyinosinic:polycytidylic acid (Poli I:C, a synthetic dsRNA), pulsed with killed PSA-positive LNCaP prostate cancer cells, and then injected in patients multiple times, subcutaneously. Phase I/II findings with DCVAC/PCa administered in combination with docetaxel/prednisone to patients with mCRPC showed a favorable safety profile, with no anaphylactic or autoimmune reactions, and a prolongation of ~6–7 months in mOS compared with the mOS predicted by standard nomograms for this population [77]. The most common AEs were fatigue, back pain, diarrhea, constipation, other gastrointestinal discomfort, paresthesia, and mild infections. All vaccine-related AEs were grade 1–2 [77]. Improvement in mOS was also observed in subsets of patients with adverse prognostic factors (i.e., increased levels of PSA, alkaline phosphatase, and lactate dehydrogenase; higher Gleason scores; presence of pain; and more extensive bone involvement). Treatment was found associated with a significant increase in the frequency of PSA-specific CD8+ T cells and a reduction in peripheral blood regulatory T cells (Tregs), which may negatively affect immune responses. However, none of the immunological parameters evaluated significantly correlated with mOS [77]. A randomized, double-blind, phase III trial (VIABLE, NCT02111577) is evaluating safety and efficacy of DCVAC/PCa in combination with docetaxel/prednisone versus docetaxel/prednisone in patients with mCRPC eligible for first-line docetaxel chemotherapy (primary endpoint: OS) [78].

PROSTVAC-V/F (rilimogene galvacirepvec/rilimogene glafolivec)

ProstVac-V/F is one of first investigational prostate cancer vaccines reaching a late development stage that was developed using a vector-based strategy. It consists of two poxviruses administered sequentially, able to express transgenes encoding a prostate cancer-associated antigen, PSA, and the costimulatory molecules B7.1, ICAM-1, and LFA-3 (PSA-CD54-CD58-CD80, PSA-TRICOM) to enhance T-cell mediated immune responses [79]. In this approach, a vaccinia-based vector is used for priming followed by 6 fowlpox-based vector boosts. Similarly to sipuleucel-T, evaluation of ProstVac-V/F in a randomized phase II study demonstrated a significant prolongation of mOS (~9–10 months) versus control empty vectors in vaccinated patients with asymptomatic/minimally symptomatic mCRPC, but no significant difference in time to disease progression [79]. Administration of ProstVac-V/F was generally well tolerated and any-grade AEs consisted most frequently of injection-site reactions with erythema (59%), fatigue (43%), nausea (21%), and pyrexia (18%). One patient discontinued due to thrombotic thrombocytopenic purpura and myocardial infarction, possibly related to treatment [80].

An independent monitoring committee recently recommended discontinuation of a randomized, double-blind, phase III trial (PROSPECT) of ProstVac-V/F with or without GM-CSF in patients with asymptomatic/minimally symptomatic mCRPC, based on futility at a pre-planned interim analysis. The trial results did not confirm the OS benefit previously observed in the phase II study [81]. Findings from combination treatment with the anti-CTLA-4 antibody ipilimumab in a phase I study showed PSA declines from baseline in 58% of the chemotherapy-naïve patients [82]. Further clinical studies are evaluating ProstVac-V/F in the adjuvant or neoadjuvant setting (with ipilimumab and/or nivolumab) and in combination with nivolumab in patients with mCRPC (NCT02933255).

ProstVac-V/F is also being investigated in patients with recurrent prostate cancer, in a triple combination with the CV301 vaccine and the bi-functional fusion protein MSB0011359C, which targets PD-L1 and transforming factor-β, to achieve stronger immune responses by combination of multiple immunotherapeutic approaches [83,84]. CV301 consists of a recombinant vaccinia viral vector (modified vaccinia Ankara-Bavarian Nordic) usually used for priming and a recombinant fowlpox viral vector for the multiple boosts. Both vectors encode the TAAs carcinoembryonic antigen (CEA) and mucin-1 (MUC-1), and the immune co-stimulatory molecules, B7-1, ICAM-1 and LFA-3 (CEA-MUC1-TRICOM) [84].

MVI-816 (pTGV-HP)

MVI-816 is plasmid DNA vaccine encoding PAP, the prostate-associated antigen targeted by sipuleucel-T [85]. Early phase I/II studies showed that intradermal administration of MVI-816 and GM-CSF in patients with minimal disease burden, stage D0, prostate cancer was associated with proliferation of PAP-specific CD4+ and/or interferon-γ producing CD8+ T-cells in some patients, and an increase in median PSA doubling time of ~2–3 months [86]. All vaccine related-AEs were grade 1–2 including, most frequently, injection-site reactions (100%), fatigue (41%), and nausea (18%); arthralgias, myalgias, and diarrhea were reported in < 15% of patients [86]. An ongoing phase Ib/expansion-cohort study is further evaluating the therapeutic potential of MVI-816 administered with adjuvant GM-CSF in combination with the anti-PD-1 antibody pembrolizumab in patients with mCRPC.

PF-06753512

PF-06753512 (PrCa VBIR) is the first cancer vaccine to be given

concurrently with two checkpoint inhibitors to break immune tolerance, amplify immune responses to self TAAs, and maintain activity in a suppressive tumor microenvironment. It consists of an intramuscular, priming vaccination with a replication-incompetent, chimpanzee adenoviral vector followed by boost vaccinations with a DNA plasmid vector by electroporation [87–89]. The adenovirus and the DNA encode three prostate cancer-associated antigens, PSA, PSMA, and PSCA, which are overexpressed in primary tumors and metastases. An anti-CTLA-4 antibody, tremelimumab, is administered with each vaccination, subcutaneously near the vaccine-draining lymph nodes, to maximize enhancement of vaccine-induced, anti-tumor immune responses. The PDGFR and VEGFR inhibitor sunitinib or an anti-PD-1 antibody (PF-06801591) is given with VBIR PF-06753512 to reduce the immunosuppressive effects of the tumor microenvironment [90–91].

Findings from preclinical studies in tumor-bearing mice and non-human primates showed that this heterologous prime/boost vaccine strategy, combined with local anti-CTLA-4 treatment, broke immune tolerance and elicited potent, polyfunctional T- and B-cell responses to the encoded, self TAAs, including anti-tumor cytolytic T cells. Administration of low-dose sunitinib effectively lowered myeloid-derived immune suppressor cells in tumors and in the periphery of tumor-bearing mice, resulting in enhanced activity of the vaccine [87–89]. A multi-center, phase I study (NCT02616185) is evaluating safety and pharmacodynamics (immune responses to prostate cancer antigens) of PF-06753512 administered with the anti-PD-1 antibody PF-06801591 in patients with biochemical relapse or mCRPC, and with sunitinib in patients with mCRPC.

CV9103 and CV9104

CV9103 and CV9104 are mRNA-based vaccines encoding multiple TAAs: PSA, PSMA, PSCA, and STEAP in C9103 and PSA, PSMA, PSCA, STEAP, PAP, and MUC1 in CV9104 [92]. The mRNA coding regions are flanked by GC-enriched sequences to increase antigen expression and complexed with protamine to enhance immunogenicity of the expressed proteins. Owing to their structure, mRNA vaccines are expected to persist in the target cells for only a few days [92]. An initial, clinical phase I/IIa study of CV9103 in CRPC showed a favorable safety profile (mostly grade 1–2 injection site reactions, fatigue, pyrexia, and chills) with induction of antigen-specific immune responses against multiple antigenic components in 15 (46%) of 33 vaccinated patients. One patient had a confirmed PSA response [93].

The coding regions for the PAP and MUC1 targets were then added to the other prostate cancer-associated antigens in a new mRNA vaccine, CV9104, to increase the range and potency of anti-cancer immune responses elicited by vaccination [92]. A randomized phase IIb study assessed safety and activity of CV9104 in chemotherapy-naive patients with oligosymptomatic/asymptomatic mCRPC. Following multiple vaccine administrations no significant improvement in OS (primary endpoint) or rPFS was observed versus placebo [94]. Grade ≥3 AEs were comparable between study arms; injection site reactions and influenza-like symptoms were reported more frequently following vaccine administration [94]. Further studies will reassess the method of administration and formulation for CV9104, and investigate a combination of this mRNA vaccine with immune checkpoint inhibitors [94].

In summary, multiple vaccines are being developed for patients with CRPC aimed at eliciting selective antitumor responses, which could be most effective at early disease stages, in the presence of low-bulk disease and an active immune system. Combination of selected vaccines with immune checkpoint inhibitors is being investigated to induce further tumor-related immune stimulation and potentially achieve effective, long-term elimination of the target cancer cells.

Conclusions

Although many of the targets and novel agents presented in this review are still being validated in clinical trials, the wide spectrum of new, potential treatment strategies being actively investigated holds promise for clinically meaningful improvements in the treatment of patients with CRPC. Findings from ongoing clinical studies of PARP inhibitors, EZH2 inhibitors, and novel therapeutic vaccines are particularly awaited, as they are aimed at mediators and cellular targets known to be strongly associated with growth and malignant progression of prostate cancer. Validation of the most sensitive tumor targets, identification of well-tolerated and active combinations, as well as selection of the optimal stage for treatment or vaccination, may all contribute to the achievement of more effective treatment options and better disease control in patients with advanced prostate cancer.

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