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Review – Prostate Cancer

Novel Molecular Targets for the Therapy of Castration-Resistant Prostate Cancer

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Abstract

Context: Improved understanding of mechanisms underlying metastatic castration-resistant prostate cancer (mCRPC) progression has led to the recognition of multiple molecular targets and advances in the therapeutic landscape. The addition of abiraterone acetate, sipuleucel-T, cabazitaxel, and denosumab to the therapeutic armamentarium and the impending addition of MDV-3100 and radium-223 underscore the importance of androgen pathway inhibition, immunotherapy, tubulin antagonism, and pathophysiology of bone metastasis.
Objective: Review the next generation of molecular targets in mCRPC.
Evidence acquisition: Medline databases were searched for >100 original articles published as of October 18, 2011, with the search terms *metastatic castration-resistant prostate cancer, targeted therapy, biologic agents*, and *immunotherapy*. Proceedings from the last 5 yr of conferences of the American Society of Clinical Oncology, American Urological Association, European Society of Medical Oncology, and the European Association of Urology were also searched. We included novel and promising drugs that have reached clinical trial evaluation.
Evidence synthesis: The major findings were addressed in an evidence-based fashion. Prospective trials and important preclinical data were analyzed.
Conclusions: mCRPC is a disease with multiple molecular drivers. Molecular pathways being targeted in ongoing phase 3 trials are androgen signaling (MDV3100, TAK700), immunoregulatory pathways (ipilimumab, Prostvac-VF-TRICOM), Src (dasatinib), Met (cabozantinib), clusterin (custirsen), and angiogenesis (aflibercept, tasquinimod). The strides made in identifying multiple other novel molecular targets offer potential opportunities for further improving outcomes.

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1. Introduction

Over the past year, multiple new systemic therapy agents have become available to treat men with metastatic castration-resistant prostate cancer (mCRPC) that provide modest but much needed benefits (Table 1). Docetaxel and cabazitaxel chemotherapy provide modest 2.5- to 3-mo extensions of median survival as first- and second-line chemotherapy, respectively [1,2]. The realization of the importance of androgen-mediated signaling led to the eventual approval of abiraterone acetate, the CYP17 and potent androgen synthesis inhibitor, in the second-line post-docetaxel setting [3]. Despite these advances, the median survival in the first-line setting of mCRPC is approximately

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Table 1 – Selected recently reported phase 3 trials in castrate-resistant prostate cancer

Molecular target Study	Line of therapy*	Experimental arm	Control arm	Median survival, mo**
VEGF Kelly et al. [26]	First	DP plus bevacizumab	DP plus placebo	22.6 vs 21.5; $p = 0.91$*
VEGF, PDGF Michaelson et al. [27]	Second	Sunitinib plus prednisone	Placebo plus prednisone	13.1 vs 12.8; $p = 0.5813$
Angiogenesis, immune mechanism Press release November 22, 2011	First	DP plus placebo	DP plus lenalidomide	No improvement
CYP17 de Bono et al. [3]	Second	Abiraterone acetate-prednisone	Placebo-prednisone	14.8 vs 10.9; $p < 0.001$
Androgen receptor Medivation [12]	Second	MDV3100	Placebo	18.4 vs 13.6; $p < 0.001$
Vitamin D receptor Scher et al. [61]	First	Weekly D plus DN-101	DP	17.8 vs 20.2; $p = 0.002$
Antigen-targeted immunotherapy Kantoff et al. [4]	First (relatively asymptomatic without visceral metastasis)	Sipuleucel-T	Placebo	25.8 vs 21.7; $p = 0.02$
Tubulin de Bono et al. [2]	First	Cabazitaxel	Docetaxel	15.1 vs 12.7; $p < 0.0001$
RANK ligand Fizazi et al. [5]	First (with bone metastasis)	Denosumab	Zoledronic acid	Time to SRE: 20·7 vs 17.1 mo; $p = 0.008$ for superiority
RANK ligand Smith [67]	First (nonmetastatic CRPC)	Denosumab	Placebo	Bone metastasis-free survival: 29.5 vs 25.2 mo; $p = 0.028$
Bone metastasis Parker [68]	First (symptomatic bone metastasis)	Radium-223	Placebo	14.0 vs 11.2; $p = 0.0022$
Endothelin receptor Press release April 21, 2011	First (with bone metastasis)	DP plus atrasentan	DP plus placebo	No improvement
Endothelin receptor Press release February 7, 2011	First (with bone metastasis)	Conventional care plus zibotentan	Conventional care plus placebo	No improvement

VEGF = vascular endothelial growth factor; DP = docetaxel plus prednisone; PDGF = platelet-derived growth factor; RANK = receptor activator of nuclear factor κB; SRE = skeletal-related event; CRPC = castration-resistant prostate cancer.
* Metastatic CRPC unless otherwise stated.
** Median overall survival unless otherwise stated.
Source: www.clinicaltrials.gov (accessed November 26, 2011).

20 mo and in the post-docetaxel setting is about 15 mo. Immunotherapy with the autologous antigen-presenting cell (APC)-based product expressing prostatic acid phosphatase-granulocyte macrophage-colony stimulating factor (PAP-GM-CSF), sipuleucel-T, extended median survival by approximately 4.5 mo in relatively asymptomatic and mostly chemotherapy-naive patients [4]. Finally, denosumab, a monoclonal antibody that targets receptor activator of nuclear factor κB ligand (RANKL), provided a modest incremental benefit, about 18%, over zoledronic acid in preventing skeletal-related events (SREs) in men with bone metastases [5].

Given the incremental benefits conferred by these recently approved agents, novel and tolerable agents are necessary to make further gains. Multiple ongoing trials are combining novel agents with first-line docetaxel-based chemotherapy (Tables 2 and 3). We review some of the most promising and emerging molecular targets in mCRPC and the efforts to develop agents against these targets.

2. Evidence acquisition

A review of the literature searching Medline and major cancer conferences for prospective trials and major preclinical and retrospective studies from the last 5 yr was performed in October 2011. The search strategy included the terms *metastatic castration-resistant prostate cancer, targeted therapy, biologic agents*, and *immunotherapy*.

3. Evidence synthesis

3.1. Biology of castration-resistant prostate cancer

Prostate cancer appears to be androgen-pathway dependent through multiple lines of therapy to varying extents, as suggested by the activity of secondary hormonal manipulations and the activity of abiraterone acetate following docetaxel. Although immunotherapy, tubulin inhibition, and osteoclast inhibition by targeting the RANKL have also yielded broad benefits, all of the hallmarks of cancer may be invoked to drive growth (Fig. 1) [6]. These include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. Increasingly recognized supportive pathways are cellular metabolism alterations, evasion of immunologic destruction, genomic instability, and inflammation by innate immune cells.

A unique fusion between the prostate-specific androgen-regulated *TMPRSS2* gene and the ETS genes *ERG, ETV1*, or *ETV4* has been described in about 50% of tumors. Although the therapeutic implications of this translocation are unclear, a tumorigenic role in early androgen-regulated prostate

Table 2 – Ongoing phase 3 trials of agents inhibiting novel targets in metastatic castrate-resistant prostate cancer

Molecular target	Line of therapy	Control arm	Experimental arm	ClinicalTrials.gov identifier
VEGF, PlGF	First	DP plus placebo	DP plus aflibercept	NCT00519285
Src, Kit, PDGFR	First	DP plus placebo	DP plus dasatinib	NCT00744497
Angiogenesis, immune mechanism	First	Placebo	Tasquinimod	NCT01234311
Clusterin	First	DP plus placebo	DP plus custirsen	NCT01188187
Clusterin	Second	DP plus placebo	DP plus custirsen	NCT01083615
CYP17 (androgen synthesis)	First	Placebo-prednisone	Abiraterone acetate-prednisone	NCT00887198
CYP17 (androgen synthesis)	First	Placebo-prednisone	TAK700-prednisone	NCT01193244
CYP17 (androgen synthesis)	Second or third	Placebo-prednisone	TAK700-prednisone	NCT01193257
Androgen receptor	First	Placebo	MDV3100	NCT01212991
Androgen receptor	Second	Placebo	MDV3100	NCT00974311
VEGFR2, Met	Third	Mitoxantrone-prednisone	Cabozantinib	Not available
CTLA-4	Second	XRT → placebo	XRT → ipilimumab	NCT00861614
CTLA-4	First	XRT → placebo	XRT → ipilimumab	NCT01057810
PSA	First	Placebo	Prostvac-Tricom plus GM-CSF*	NCT01322490
Tubulin	First	Docetaxel	Cabazitaxel 20 mg/m^2 or 25 mg/m^2	NCT01308567

VEGF = vascular endothelial growth factor; PlGF = placental growth factor; DP = docetaxel plus prednisone; PDGFR = platelet-derived growth factor receptor; CTLA = cytotoxic T lymphocyte antigen; PSA = prostate-specific antigen; GM-CSF = granulocyte-macrophage colony-stimulating factor.
Source: www.clinicaltrials.gov (accessed November 26, 2011).
* Poxvirus-carrying PSA and immune stimulatory Tricom.

Table 3 – Agents against novel targets undergoing phase 1/2 trials in metastatic castrate-resistant prostate cancer

Molecular targets	Regimen	ClinicalTrials.gov identifier
VEGF, mTOR	Docetaxel plus bevacizumab plus everolimus	NCT00574769
	Bevacizumab plus temsirolimus	NCT01083368
mTOR	Temsirolimus	NCT00919035
	Docetaxel plus temsirolimus	NCT01206036
	Bicalutamide plus or minus everolimus	NCT00814788
	Carboplatin plus everolimus	NCT01051570
PI3K	PX-866	NCT01331083
	BKM-120	NCT01385293
HGF/Met	Mitoxantrone with or without AMG-102	NCT00770848
VEGF receptor	Pazopanib	NCT00945477
VEGF and FGF receptors	Dovitinib	Not available
PDGFR-α	Mitoxantrone with or without IMC-3G3	NCT01204710
$\alpha_v\beta_3$ integrin	EMD-525797	NCT01360840
Methylation	Docetaxel plus azacitidine	NCT00503984
HDAC	Valproic acid	NCT00670046
	SB-939	NCT01075308
mTOR, HDAC	Temsirolimus plus vorinostat	NCT01174199
CYP17	Docetaxel plus TAK-700	NCT01084655
AR	ARN-509	NCT01171898
	EZN-4176 (ASO)	NCT01337518
	ODM-201	NCT01317641
Angiogenesis (CD105)	TRC-105	NCT01090765
eIF-4E	Docetaxel plus ISIS EIF4E Rx	NCT01234025
Topoisomerase II	AEZS-108	NCT01240629
Phosphatidylserine	Cabazitaxel plus bavituximab	NCT01335204
Polyamine oxidase	APC-100	NCT01436214
Hsp-27	OGX 427	NCT01120470
Hsp-90	STA-9090	NCT01270880
Src	Placebo vs saracatinib	NCT01267266
Tubulin	Tesetaxel (oral taxane)	NCT01296243
	Patupilone vs docetaxel	NCT00411528
Aurora kinase A	Docetaxel plus MLN-8237	NCT01094288
PSMA targeting radioimmunotherapy	177Lu-CYT-500	NCT00441571
	Ketoconazole with or without 177Lu-J591	NCT00859781
	Docetaxel plus 177Lu-J591	NCT00916123
PSMA targeting autologous dendritic cell or T-cell based immunotherapy	BPX-101 (DC vaccine with inducible CD40 and in vivo activation)	NCT00868595
	Designer T cells	NCT00664196
NY-ESO	Peptide vaccine	NCT00616291
Proteasome	Mitoxantrone with or without bortezomib	Not available

VEGF = vascular endothelial growth factor; mTOR = mammalian target of rapamycin; HGF = hepatocyte growth factor; PDGFR = platelet-derived growth factor; HDAC = histone deacetylase; AR = androgen receptor; AEZS-108 = doxorubicin-LHRH conjugate; PSMA = prostate-specific membrane antigen.
Source: www.clinicaltrials.gov (accessed October 31, 2011).

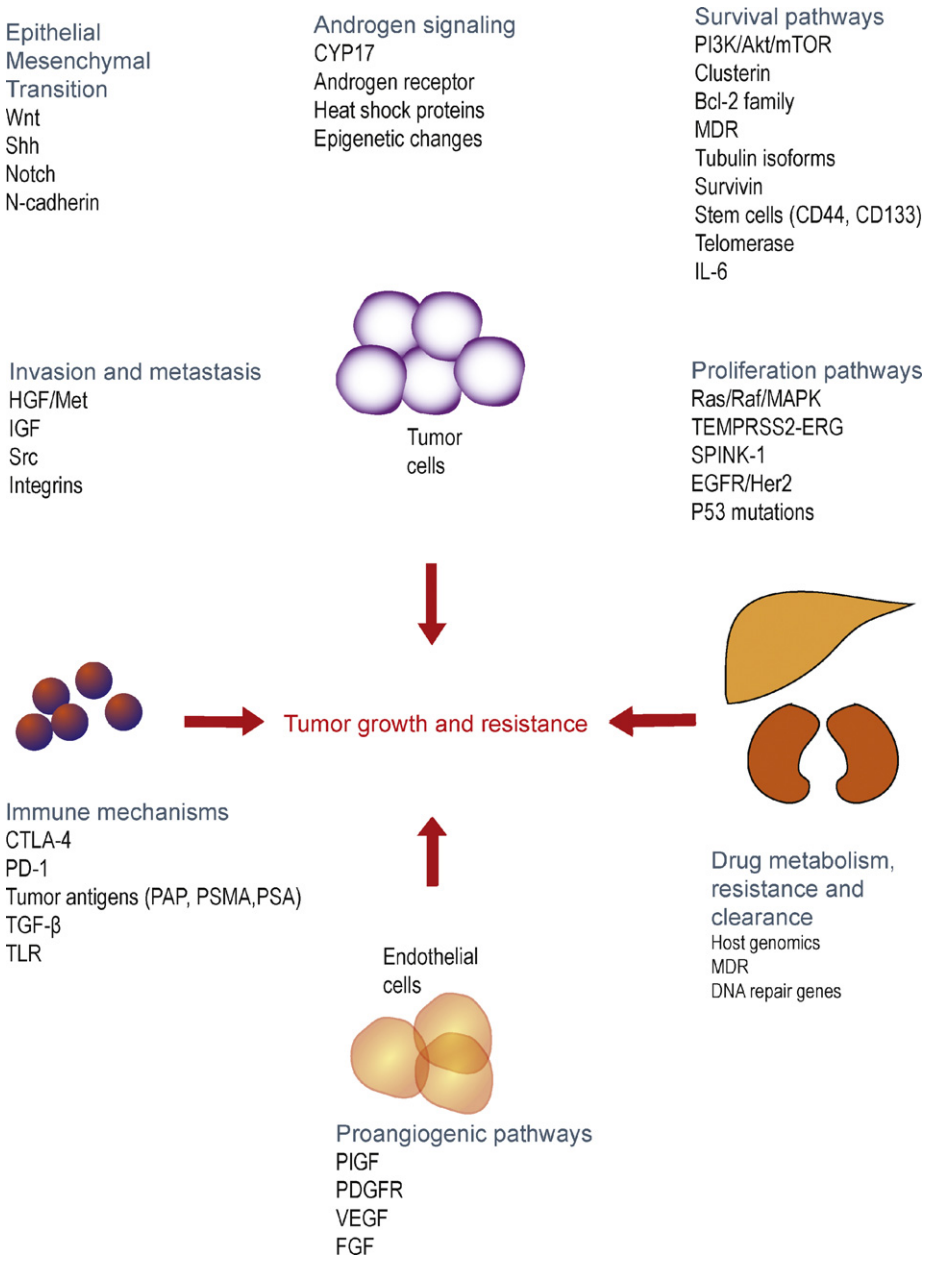

Fig. 1 – Molecular targets and mechanisms of resistance.
CTLA = cytotoxic T lymphocyte antigen; EGFR = epidermal growth factor receptor; FGF = fibroblast growth factor; HGF = hepatocyte growth factor; IGF = insulinlike growth factor; IL = interleukin; MDR = multidrug drug resistance; mTOR = mammalian target of rapamycin; PAP = prostate acid phosphatase; PD = programmed death; PDGFR = platelet-derived growth factor receptor; PlGF = placental growth factor; PSA = prostate-specific antigen; PSMA = prostate-specific membrane antigen; TGF = transforming growth factor; TLR = toll-like receptor; VEGF = vascular endothelial growth factor.

cancers appears likely [7]. A systems biology approach may identify the most relevant signaling pathways (as opposed to discrete molecules) and enable focused study of the pathophysiology of prostate cancer. For example, one study identified four pathways that appeared central including c-Myc, p53, androgen receptor (AR), and prostate-specific antigen (PSA) [8]. The Catalogue of Somatic Mutations in Cancer database maintained by the Wellcome Trust Sanger Institute has identified common gene alterations (with the caveat that these were mostly localized disease), which may help focus on the appropriate targets for therapy (Fig. 2).

3.2. *Data supporting the role of novel molecular targets*

3.2.1. *Androgen pathway*

3.2.1.1. Androgen synthesis. The role of persistent androgen-axis signaling mediated by adrenal, testicular, and intratumoral androgen synthesis, and AR amplification and mutations in driving tumor growth is now well recognized.

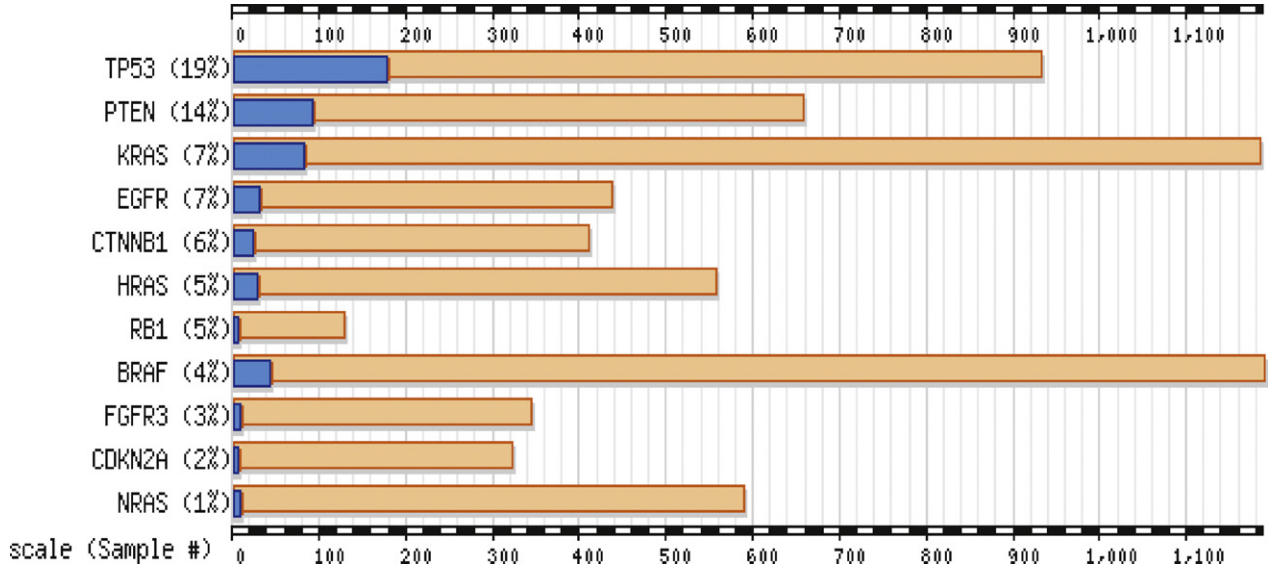

Fig. 2 – Significantly mutated genes in prostate cancer. The mutation data were obtained from the Sanger Institute Catalogue of Somatic Mutations in Cancer Web site (http://www.sanger.ac.uk/cosmic, accessed October 31, 2011). The red bars represent the total number of examined tumor samples, and the blue bars represent the number of tumor samples with mutated genes. Reprinted with permission from Cancer Research UK [71].

The CYP17 enzymes mediating androgen synthesis, 17 α-hydroxylase and C17,20-lyase, are validated targets based on the improved outcomes observed with abiraterone acetate following docetaxel (Table 1) [3]. An ongoing phase 3 trial is evaluating abiraterone acetate in the chemotherapy-naive mCRPC setting (Table 2). On the heels of abiraterone acetate, TAK700, a CYP17 inhibitor with potentially greater 17,20 lyase selectivity (ie, for androgen as opposed to corticosteroid synthesis), is being developed (Table 2) [9]. Currently, TAK-700 is being evaluated in two separate placebo-controlled phase 3 trials (with prednisone in both arms) of men with progressive mCRPC who are either chemotherapy naive or post-docetaxel. Studies are also starting to elucidate mechanisms of resistance to abiraterone, which may be mediated by amplification of CYP17 (indicating a potential role for dose escalation of abiraterone) and AR splice variants [10]. Inhibitors of the conversion of *d*-cholesterol to *d*-pregnenolone (HE3235), 17βHSD5 inhibitors (ASP9521), and steroid sulfatase inhibitors (irosustat) also inhibit androgen synthesis and are undergoing evaluation.

3.2.1.2. Androgen receptor. MDV 3100 is a novel AR antagonist that binds AR with a higher affinity than bicalutamide and inhibits its nuclear translocation. In a phase 1/2 trial of 140 patients with progressive mCRPC, antitumor activity was noted including declines in serum PSA ≥50% in 56% of patients, responses in soft tissue in 22%, stabilization of bone disease in 56%, and conversion from unfavorable to favorable circulating tumor cells (CTCs) in 49% [11]. These data led to placebo-controlled phase 3 trials (without prednisone) in chemotherapy-naive and post-docetaxel patients with mCRPC (Table 2). In a recent press release, a 4.8-mo advantage in median survival was reported in the post-docetaxel trial (18.4 vs 13.6 mo; hazard ratio [HR]: 0.631; $p < 0.001$) [12]. ARN-509 has a similar mechanism of activity and is undergoing early evaluation (Table 2). TOK-001, a dual inhibitor of CYP17 and AR, is undergoing evaluation in chemotherapy-naive patients (Table 2). EPI-001, a small molecule inhibitor of the amino-terminal domain of AR, which confers transcriptional activity, demonstrated substantial preclinical activity warranting clinical development of this class of agents [13]. Interestingly, a robust transcription-based AR activity signature appears to reflect hormone status and intraprostatic dihydrotestosterone levels [14]. AR activity was high in local untreated prostate cancer and decreased after neoadjuvant androgen-deprivation therapy (ADT) and in mCRPC. Decreasing AR activity correlated with increasing Src activity and sensitivity to dasatinib.

3.2.1.3. Epigenetic pathways. Epigenetic mechanisms (methylation, histone deacetylation) can modulate gene expression by altering chromatin structure. For example, an open chromatin structure induced by hypomethylation can facilitate gene transcription, whereas a closed structure inhibits transcription. AR binds with androgen response elements and recruits cofactors such as histone acetyltransferases and histone deacetylases (HDACs) and leads to transcription. Inhibition of HDAC activity by LAQ824 preclinically depleted AR partly by Hsp90 acetylation resulting in dissociation of the Hsp90-AR complex and proteasome-mediated degradation of AR [15]. Vorinostat, a small molecule inhibitor of class I and II HDACs, did not demonstrate activity in mCRPC following docetaxel; panobinostat, a pan-deacetylase inhibitor, is undergoing evaluation (Table 2) [16]. In one phase 2 trial, azacitidine, a hypomethylating agent, appeared to slow the pace of PSA doubling in chemotherapy-naive men with mCRPC, which correlated with plasma DNA hypomethylation [17]. Further evaluation is ongoing in combination with docetaxel in progressive disease following docetaxel (Table 2). The early identification of antitumor activity

may be problematic when investigating agents that yield epigenetic activity, given that proliferative activity of tumor cells may be required for progressive epigenetic changes. This unique mechanism of activity may lead to delayed benefits, reminiscent of the phenomenon observed with immunotherapy. Hence the adoption of intermediate end points similar to the immune-related response criteria may be warranted [18].

3.2.1.4. Chaperone proteins. Complexes of AR and chaperone proteins, for example, heat shock protein (HSP)-90, protect AR and other key molecules (eg, Akt, Her2), and degradation of such chaperones by ansamycin antibiotic analogs was demonstrated to confer antitumor activity preclinically [19]. The AR-HSP-90 complex maintains AR in a high-affinity ligand-binding conformation. Unfortunately, HSP-90 inhibition with a novel agent, IPI-504, demonstrated minimal antitumor activity in an unselected population and was associated with unacceptable toxicities [20]. Other HSP inhibitors are undergoing evaluation, and a more focused development based on predictive biomarkers may be warranted (Table 2).

3.2.2. Immune system

Sipuleucel-T is the first therapeutic vaccine demonstrated to improve outcomes in an advanced malignancy [4]. Sipuleucel-T consists of APCs cultured with a fusion protein composed of PAP and GM-CSF. In the phase 3 IMPACT trial including men with relatively asymptomatic mCRPC, the median survival improved when compared with placebo (25.8 mo vs 21.7 mo; HR: 0.77; $p = 0.02$). The toxicity profile was excellent, and the time to progression was similar in both groups and was not accompanied by measurable antitumor effect. These results validate the efficacy of immunotherapy and provide the impetus for its further investigation. Earlier deployment of sipuleucel-T in hormone-sensitive metastatic disease is being evaluated in a phase 3 trial. A second-generation autologous APC-based immunotherapy, BPX-101, has preliminarily demonstrated promising efficacy [21]. Prostvac-VF is an example of a heterologous prime/boost vaccination strategy that exploits the immunogenicity of recombinant poxviral vectors (vaccinia vector and fowlpox vector), each encoding transgenes for PSA and TRICOM. TRICOM consists of costimulatory molecules, intercellular addition molecules-1 (CD54), B7.1 (CD80), and leukocyte function-associated antigen-3 (CD58). In a double-blind randomized phase 2 trial of patients with chemotherapy-naive minimally symptomatic mCRPC, Prostvac-VF improved median survival (25.1 vs 16.6 mo; $p = 0.0061$), which provided the rationale for a phase 3 trial (Table 2). Conversely, disappointing results were observed with GVAX, an allogeneic cell-line–based vaccine engineered to secrete GM-CSF, both alone or in combination with docetaxel. Designer autologous T cells targeting tumor antigens (eg, prostate-specific membrane antigen [PSMA]) and peptide vaccines against tumor antigens such as NY-ESO and MUC1 are in the early stages of development (Table 3).

The T lymphocyte checkpoint, cytotoxic T lymphocyte antigen (CTLA)-4, has emerged as a major target and has been validated in advanced melanoma. Based on preliminary evidence for radiotherapy enhancing the activity of subsequent immunotherapy, two placebo-controlled phase 3 trials are evaluating ipilimumab following brief radiation to a metastatic site in the chemotherapy-naive and post-docetaxel settings (Table 2). CTLA-4 inhibition is associated with potentially life-threatening autoimmune phenomena such as enterocolitis, hypophysitis, and dermatitis. Programmed death (PD)-1, a member of the extended CD28/CTLA-4 family of T-cell regulators, and its ligands negatively regulate immune responses. PD-1 inhibitors appear to have a more favorable toxicity profile and may warrant evaluation in CRPC [22]. Toll-like receptor agonists are also emerging as agents that may play a supportive role in bolstering T-cell response [23]. In established malignancy, transforming growth factor (TGF)-β signaling may induce angiogenesis, suppress immune surveillance, enhance AR translocation to the nucleus, and yield castration-resistance. One preclinical study demonstrated antiangiogenic and antitumor activity for the inhibition of TGF-β [24]. The lutetium-177–labeled anti-PSMA monoclonal antibody, J591, can be administered in higher doses, with comparatively less myelosuppression and little nonhematologic toxicities [25]. A randomized phase 2 trial is evaluating this agent in nonmetastatic CRPC with biochemical progression (Table 3).

3.2.3. Angiogenesis

Despite encouraging data from initial phase 2 trials, subsequent phase 3 trials have not demonstrated a survival benefit in patients receiving vascular endothelial growth factor (VEGF) inhibitors. Bevacizumab in combination with docetaxel-prednisone or sunitinib in combination with prednisone following docetaxel have not improved survival [26,27]. Broader inhibition of proangiogenic molecules with aflibercept (VEGF Trap), a recombinant humanized fusion protein consisting of receptors for the VEGF extracellular domains and the Fc portion of human immunoglobulin (Ig)G1, which inhibits all isoforms of VEGF as well as placental growth factor (PlGF), is being investigated in a phase 3 trial (Table 2). Dovitinib, a small molecule tyrosine kinase inhibitor (TKI) that targets multiple angiogenic pathways (VEGF, platelet-derived growth factor [PDGF], and fibroblast growth factor [FGF]), is being studied in a trial that is supported by preclinical evidence for the importance of the FGF pathway (Table 2) [28]. The combination of lenalidomide with docetaxel was assessed in the phase 3 MAINSAIL trial, based on the angiogenesis and immune modulating properties of lenalidomide (Table 1). Unfortunately, according to a press release, this trial was stopped due to an excess of adverse events and a lack of improved outcomes. Tasquinimod, an oral quinoline-3-carboxamide derivative with antiangiogenic (by upregulating thrombospondin) and immunomodulatory properties, partly by targeting S100A9 (MRP-14), which is expressed on myeloid-derived suppressor cells, demonstrated 6-mo progression-free survival (PFS) improvement compared with placebo (69% vs 37%) in conjunction with a favorable toxicity profile [29]. A phase 3 trial is

evaluating tasquinimod in chemotherapy-naive mCRPC (Table 2).

3.2.4. Invasion and metastasis

Several molecules involved in tumor cell invasion and metastasis have been recognized (Fig. 1). Dasatinib, a small molecule inhibitor of the Src family of nonreceptor protein tyrosine kinases (SFKs), in addition to other molecules (notably Kit and platelet-derived growth factor receptor), yielded stable disease in 19% of patients at 24 wk accompanied by reduction in bone turnover markers [30]. The combination of docetaxel and dasatinib was safe and active, which led to an ongoing phase 3 trial (Table 2). Saracatinib, another SFK inhibitor, is undergoing phase 2 evaluation (Table 3).

The hepatocyte growth factor (HGF) and c-Met axis have tumorigenic activity partly by downstream activation of the Ras/MEK pathway and PI3K/AKT pathway. Interestingly, AR appears to suppress c-Met transcription, and c-Met expression is upregulated by castration in animals. Preclinical activity has been demonstrated for selective Met inhibitors, which may also be synergistic with hormonal therapy [31,32]. Cabozantinib (XL-184), an inhibitor of c-MET and VEGF receptor tyrosine kinases, yielded promising symptomatic and bone scan responses in a phase 2 trial [33]. In this trial, patients with stable disease after 12 wk were randomized to receive cabozantinib or placebo. At 12 wk, disease control was seen in 68% of patients. Among the 31 patients who were randomly assigned to placebo (17 patients) or cabozantinib (14 patients), median PFS was significantly longer with cabozantinib (21 vs 6 wk; $p = 0.0007$). Of 108 patients with bone scans, 21 (19%) showed complete resolution and 61 (56%) showed partial resolution. Adverse events were typical for small molecule VEGF inhibitors (ie, fatigue, palmar-plantar erythrodysesthesia, and hypertension). A phase 3 trial is planned to further develop cabozantinib (Table 2). AMG-102, a monoclonal antibody that binds HGF, is being evaluated in a randomized phase 2 trial combined with mitoxantrone and prednisone (Table 3).

Blockade of insulinlike growth factor-1 receptor (IGF-IR) by a monoclonal antibody, figitumumab, in combination with docetaxel, appeared tolerable and active [34]. Of 18 patients with mCRPC, 3 had confirmed objective responses, and 8 had disease stabilization lasting $\geq$6 mo. Similarly, cixutumumab, another monoclonal antibody, yielded disease stabilization $\geq$6 mo in about 30% of chemotherapy-naive patients [35]. A randomized phase 2 trial is evaluating the impact of combining cixutumumab, an IGF-1R monoclonal antibody with combined ADT for metastatic hormone-sensitive disease. Integrins are cell adhesion receptors that regulate the attachment of epithelial cells to the basement membrane and enhance invasion, migration, and angiogenesis. Preclinical activity has been demonstrated for α (v)-β-3 integrin inhibitors, although one clinical trial demonstrated no activity in nonmetastatic CRPC [36]. Notch signaling has also been implicated in stromal–epithelial communications, and one preclinical study demonstrated that the knockdown of Notch1 inhibits invasion [37]. A phase 2 randomized trial is investigating bicalutamide alone or with a γ-secretase/Notch signaling inhibitor, RO4929097, in patients with rising PSA after local therapy (NCT01200810).

3.2.5. Epithelial-mesenchymal transition

Epithelial-mesenchymal transition (EMT) appears to be caused by decreases in epithelial genes such as E-cadherin and increases in mesenchymal genes such as vimentin and N-cadherin. These alterations may be driven by transcription factors including Snail, Twist, and ZEB. Numerous signaling pathways are potential mediators of EMT including HGF-Met, IGF-1, Hedgehog (Hh), Wnt, PDGF, FGF, and TGF-β. These changes alter cell morphology and lead to loss of polarity, an invasive and migratory phenotype, and perhaps cells with stem cell characteristics. Interestingly, TMPRSS2/ERG overexpressing cells underwent EMT [38]. Hh inhibitors, proteasome inhibitors, and N-cadherin inhibitors have demonstrated preliminary activity, which has prompted clinical trials (Table 3) [39–41]. The upregulation of pAkt following neoadjuvant bortezomib preceding prostatectomy suggests that the combination of bortezomib and Akt inhibitors may warrant investigation [42].

3.2.6. Tumor initiating or stem cells

A growing body of evidence indicates that tumor cells exhibit a hierarchy of cell populations that may owe their origin to stem cells that display self-renewal characteristics and maintain the tumor. As previously described, pathways that promote cell survival, invasion, and EMT may be partly responsible for maintaining stem cells. One study demonstrated that stem cells express CD44 + /α2β1hi/CD133 + and appear to constitute only 0.1% of the tumor, which may also represent a molecular target for therapy [43]. In another study, expression of CD133 corresponded to the loss of AR. Expression of CXCR4 was also detected in CD133(+) cancer cells and the inhibition of CXCR4/SDF-1-mediated chemotaxis was inhibited in vitro by anti-CXCR4 antibodies [44].

3.2.7. Cell survival pathways

Loss of function of phosphatase and tensin homolog (PTEN) appears to play an important role in progression, and a sequencing study of 218 prostate cancers found PTEN-inactivating mutations in 4% of primary and 42% of metastatic tumors, although when examining the entire PI3K pathway, deregulation was detected in 42% of all primary tumors and 100% of metastases [45]. Everolimus-induced mitogen-activated protein kinase (MAPK) activation occurred via a feedback loop depending on the S6K-PI3K-Ras pathway, and inhibition of the MAPK pathway enhanced the antitumor activity of everolimus [46]. These data may explain the marginal clinical activity observed for single-agent mammalian target of rapamycin complex 1 (mTORC1) inhibition and provide a rationale to investigate a combination approach with mTORC1 and MAPK inhibitors. The combination of docetaxel and BEZ-235 appeared significantly more effective than single-agent therapy, perhaps by targeting different

subsets of cells (ie, differentiated and tumor-initiating cells) [47]. Additionally, data indicate cross-talk between the PI3K and AR pathways, and the preclinical combination of AR and PI3K inhibitors (MDV-3100 and BEZ-235, respectively) induced maximal PTEN-negative tumor regressions [48]. Inhibition of HER2/3 also abolished the upregulation of AR induced by PI3K inhibition.

Bcl-2 overexpression appears to play a role in the onset of castration resistance, as well as resistance. Unfortunately, both oblimersen sodium, an antisense oligonucleotide (ASO) for Bcl-2 and AT-101, a small molecule antagonist of the Bcl-2 family, did not improve outcomes when combined with docetaxel [49,50]. Further development of more potent Bcl-2 antagonists with a focus on individualized treatment may be warranted because the high clinical risk group appeared to enjoy differential benefits with AT-101. Conversely, OGX-011 (custirsen), a second-generation antisense complementary to clusterin, which promotes cell survival partly by inhibiting Bax, demonstrated preliminary evidence for improved survival in combination with docetaxel, which has led to an ongoing phase 3 trial (Table 2) [51]. A survivin inhibitor, YM155, displayed prolonged stable disease (≥18 wk) in 25% of post-taxane patients [52]. Telomerase attaches telomeric DNA to the ends of chromosomes, prevents chromosome degradation, and overcomes replicative senescence, and it may represent a promising target for further investigation. The combination of methylseleninic acid and bicalutamide suppressed telomerase and conferred apoptotic activity, which was reversed by restoring hTERT [53].

3.2.8. Proliferation signaling

Ligand-mediated proliferative signaling through the Ras-Raf-MAPK pathway appears to play a role in the pathobiology of mCRPC, partly by downregulating AR [54]. Simultaneous inhibition of the ERK1/2 and PI3K pathways may be required to exert a synergistic efficacy [55]. The commonly upregulated PI3K pathway may explain the poor response to EGFR or Her2 inhibitors [56,57]. ETS-mediated DNA damage, cell proliferation, and invasion may be potentiated by PARP1, and inhibition of PARP1 inhibited ETS-positive, but not ETS-negative, prostate cancer xenografts [58]. Conversely, serine peptidase inhibitor, Kazal type 1 (SPINK1) expression appeared to be amplified in a subset of ETS-negative aggressive disease, which was partially mediated by its collaboration with EGFR. In a SPINK1-positive murine model, monoclonal antibodies to either SPINK1 or EGFR (cetuximab) suppressed growth, and the combination of both agents appeared additive [59]. One randomized phase II trial did not demonstrate improved overall outcomes by combining cetuximab with mitoxantrone-prednisone following docetaxel, although intriguingly, those who developed a skin rash exhibited significantly better outcomes [60]. A notable failure has been the combination of weekly docetaxel and DN-101 (a potent calcitriol analog), which was purported to inhibit proliferation and induce apoptosis but was associated with a decrement in survival compared with conventional docetaxel every 3 wk plus prednisone in a phase 3 trial [61].

3.2.9. Delivery of downregulated tumor suppressor genes

Although most agents attempt to inhibit an upregulated molecule, the introduction of repressed tumor suppressor genes may address the more commonplace and fundamental genetic aberrations (Fig. 2). Deletions and mutations in tumor suppressor genes appear more common than activating mutations of oncogenes, and these alterations engender multiple downstream amplifications of tumorigenic molecules. Indeed, p53 alterations appear to be the most common somatic tumor aberration (Fig. 2). Hence a greater focus on introducing the function of missing or downregulated molecules may be entailed. For example, the intratumoral introduction of p53 or its collaborators (eg, GLIPR1) by adenoviral vectors has demonstrated promise [62].

3.2.10. DNA replication and mitosis: novel chemotherapeutic agents

The activity of cabazitaxel, a tubulin-stabilizing taxoid in patients who were relatively heavily pretreated with docetaxel, suggests that the continued investigation of chemotherapy, particularly tubulin antagonists, may yield benefits (Table 2) [2]. Docetaxel retreatment has activity in patients who were effectively pretreated with docetaxel [63]. Conversely, taxanes may have activity partly by targeting AR. One ongoing phase 3 trial attempts to optimize first-line chemotherapy and compares two doses of cabazitaxel with docetaxel. Cabazitaxel may be able to overcome resistance conferred by the multidrug resistance protein and penetrate the blood-brain barrier more effectively. Carboplatin has demonstrated activity and appeared active in combination with docetaxel in progressive disease following docetaxel alone [64]. Potentially, tumors with defective DNA damage repair (eg, decreased ERCC-1) may be more sensitive to platinums. Ixabepilone, an epothilone, and eribulin, a halichondrin-B analog, have both demonstrated antitumor activity similar to docetaxel [65,66]. Patupilone, an epothilone, is being compared with docetaxel in a randomized phase 2 trial (Table 3).

3.2.11. Molecular targets involved in the pathophysiology of bone metastasis

Zoledronic acid historically has diminished SREs by 24%, and denosumab, a subcutaneously administered RANKL antagonist monoclonal antibody, decreased the risk by a further 18% [5]. In a phase 3 trial, the median time to the first on-study SRE was 20.7 mo with denosumab compared with 17.1 mo with zoledronic acid (HR: 0.82; 95% confidence interval, 0.71–0.95; $p = 0.0002$ for noninferiority; $p = 0.008$ for superiority). Denosumab has extended the median time to bone metastasis by approximately 4 mo (29.5 vs 25.2 mo; $p = 0.028$) in nonmetastatic CRPC and PSA ≥8 ng/ml or PSA doubling time ≤10 mo [67]. The shorter range but more potent α-emitting radiopharmaceutical, radium-223, reduced pain and extended median survival (14.0 vs 11.2 mo; HR: 0.699; $p = 0.0022$) in men with symptomatic bone metastasis in a phase 3 trial, and regulatory approval is anticipated [68]. Conversely, phase 3 trials evaluating the endothelin-A receptor antagonists atrasentan and zibotentan have been disappointing according to preliminary press releases. Src kinase inhibition

confers bone protection, and results from the phase 3 trial combining dasatinib with docetaxel-based chemotherapy are awaited (Table 2).

3.2.12. Strategies for further drug development

The targeting of a single molecule or pathway may yield limited benefits, and rational combinations of novel biologic agents (based on compelling preclinical rationale) and the earlier application of agents (eg, sipuleucel-T) may yield the most optimal benefits. The development of novel agents needs to proceed in conjunction with the discovery of biomarkers predictive of efficacy (eg, CTC profiling, plasma-based markers, and functional imaging). Concurrently, optimal intermediate clinical end points require validation in the setting of biologic agents. Favorable CTC counts 3–12 wk after beginning therapy appear promising at least in the setting of second-line abiraterone and may complement time to event end points recommended by the Prostate Cancer Clinical Trial Working Group-2 [69,70]. Immune-related response criteria allow the assessment of tumor burden as a continuous variable and address the delayed activity of immunotherapy [18].

The lack of one dominant molecular driver across all tumors suggests that no single class of agents is likely to confer broad and substantial benefits across all patients with mCRPC. Innovative clinical trial designs (eg, using genomic characterization of the tumor), followed by adaptive randomization, may be warranted similar to the Biomarker-Integrated Approaches of Targeted Therapy for Lung Cancer Elimination non–small cell lung cancer trial. Alternatively, smaller proof of concept trials driven by biomarkers may be justified. However, obtaining tumor tissue from patients with metastatic disease prior to and during the trial remains a significant challenge and at times a deterrent, if mandated, to trial enrollment and participation. The paradigm of neoadjuvant therapy preceding prostatectomy may provide easy access to baseline and post-therapy tumor tissue and an early signal of activity and mechanisms of resistance, with the caveat that activity in early disease may not translate to benefits in castration-resistant disease. Another challenge is in conducting trials investigating rational combinations of drugs but from different manufacturers, although some trials in other malignancies indicate that a compelling scientific rationale may overcome this barrier.

4. Conclusions

Although multiple new drugs have recently been approved for the treatment of mCRPC, improvement in overall survival remains modest at best, with all patients eventually experiencing disease progression and early mortality. In addition to immunotherapy and androgen pathway signaling, multiple other molecular targets promise to provide the next generation of advances. In recent years, the simultaneous development of novel and more potent classes of drugs targeting these pathways has emerged. A commitment to trials and close collaboration between basic and clinical investigators is imperative to yield further advances.

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Study concept and design: Sonpavde.
Acquisition of data: Agarwal, Sternberg, Sonpavde.
Analysis and interpretation of data: Agarwal, Sternberg, Sonpavde.
Drafting of the manuscript: Agarwal, Sternberg, Sonpavde.
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