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REVIEW ARTICLE

Biomarkers for the Management of Castration-Resistant Prostate Cancer: We Are Not There Yet

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Abstract In recent years, there has been a marked increase in the number of approved therapies that increase survival of patients with castration-resistant prostate cancer. Current treatment guidelines provide therapeutic management recommendations, but these are primarily based on clinical factors such as performance status or site of metastasis (bone vs. visceral), and not on underlying molecular or cellular features of disease that may predict response. The ability to tailor treatment based on molecular or cellular features of disease could potentially reduce the occurrence of unnecessary side effects and ineffective treatments, and thereby reduce both direct and indirect medical costs. As such, it is important to identify and validate new prognostic and predictive molecular biomarkers that can be used to direct cancer treatment. This review will focus on existing and potential biomarkers in the context of castration-resistant prostate cancer management and discuss the need for continued discovery and validation of new biomarkers and biomarker panels for prostate cancer.

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Key Points

A number of new therapeutic options for the management of castration-resistant prostate cancer have become available.

However, the availability of validated biomarkers to aid in treatment decisions or to serve as surrogate endpoints in clinical trials are limited to prostate-specific antigen and circulating tumor cells.

Other potential biomarkers that have been explored include androgen receptor variants, mutated DNA repair genes, *TMPRSS2-ERG* rearrangements, PTEN loss, and interleukin-6.

1 Introduction

Among men in the USA, prostate cancer (PCa) is the most commonly diagnosed noncutaneous cancer and remains the second-leading cause of cancer-related death [1]. While 5-year survival rates for men with local or regional PCa, which comprise 93% of newly diagnosed cases, are close to 100% [2], the prognosis for those with metastatic PCa at diagnosis or who experience tumor recurrence after definitive local therapy (i.e., radical prostatectomy or radiation therapy) and go on to develop castration-resistant PCa (CRPC) is much more dismal.

In recent years, there has been a marked increase in the number of approved therapies that increase survival in CRPC. These include docetaxel and cabazitaxel (chemotherapy), abiraterone acetate and enzalutamide (hormonal therapy), sipuleucel-T (immunotherapy), and radium-223 (bone microenvironment-targeting agents) [3, 4]. Given this increase in the number of available treatment options, guidance on optimal treatment choices and sequencing for each patient is needed [3]. For a few years, docetaxel, the first of the above-mentioned therapies to be approved for use in CRPC, had been the only new option for patients, but now both abiraterone and enzalutamide are approved for use in CRPC patients who have either progressed after docetaxel therapy or are docetaxel-naïve. However, cross-resistance between therapies has been demonstrated. For example, the efficacy of enzalutamide may be blunted when administered after a patient has already received abiraterone, docetaxel, or both [5–7]. In contrast, alternative sequencing of abiraterone and docetaxel in men with CRPC resulted in no significant differences in clinical outcomes [5, 8]. So, how does a physician choose the appropriate treatments and their sequence for each patient? Currently, recommendations for treatment sequencing have been provided by a number of societies, including the American Urologic Association (AUA) [9], the National Comprehensive Cancer Network (NCCN) [10], the American Society of Clinical Oncology (ASCO) [11], the European Society for Medical Oncology (ESMO) [12], and the European Association of Urology (EAU) [13], but these are primarily based on clinical factors such as performance status and site of metastasis (bone vs. visceral), and not on underlying molecular or cellular features of disease that may predict response. The ability to tailor treatments based on molecular or cellular features of disease could potentially reduce the occurrence of unnecessary side effects and ineffective treatments, and thereby reduce both direct and indirect medical costs [14]. As such, it is important to identify and validate new prognostic and predictive molecular biomarkers that can be used to direct cancer treatment. This review will focus on existing and potential biomarkers in the context of CRPC management and discuss the need for continued discovery and validation of new biomarkers and biomarker panels for PCa.

2 The Need for Biomarkers to Facilitate Personalized Medicine in CRPC Management

The USA Food and Drug Administration (FDA) defines a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or biological responses to a therapeutic intervention" [15]. There are four types of biomarkers, according to the FDA: 1) prognostic markers, which indicate risk for disease recurrence or progression and provide information regarding the natural history of a disease in the absence of intervention; 2) predictive markers, which can help predict response (positive or negative) to a particular treatment; 3) pharmacodynamic markers, which provide information about certain biologic responses that have occurred during a therapeutic intervention, and can be treatment-specific or more broadly related to the disease; and 4) surrogate endpoints, which are biomarkers intended to substitute clinical efficacy endpoints, such as overall survival (OS) [15]. For PCa, there are few surrogate endpoints, as this qualification requires rigorous scientific evaluation and statistical confirmation. The Prentice criteria are widely used to determine surrogacy; the surrogate endpoint must be statistically associated with the clinical outcome and also capture the net effect of treatment on that clinical outcome [16]. To date, only prostate-specific antigen (PSA) decline and circulating tumor cell (CTC) enumeration have met the Prentice criteria for surrogacy for OS (discussed in detail in the sections below). Biomarker development comprises multiple steps: discovery, validation, qualification, and clinical implementation [17]. Opportunities for biomarker development arise at different stages of carcinogenesis, including biomarkers for altered gene expression, biomarkers for altered protein expression, or biomarkers that are visualized at the cellular level.

Personalized or targeted therapy has become more common in clinical practice for many other cancer types; however, it is not as common in PCa management [18]. Biomarker panels have been developed for a handful of cancer types, such as CollabRx (lung cancer, colorectal cancer, and melanoma) and Oncotype Dx (breast and colon cancer). Recently, two genetic biomarker panels, Prolaris cell cycle progression score and Oncotype DX genomic prostate score, which have been designed and validated for use in localized PCa, are available for PCa risk stratification at diagnosis and are now part of the NCCN guidelines [10], although their use has not been widely adopted. The success of these markers in localized disease is due to the fact that this signal can be derived from a local biopsy or radical prostatectomy specimen. The problem becomes more complex for those patients with advanced CRPC. The heterogeneity of germline and somatic changes seen in the primary and metastatic lesion may render sampling of a single lesion for biological markers less applicable to the treatment of a patient. Changes also potentially can occur due to the biological effects of various treatments, which can include up-regulation of genes as well as clonal selection. Thus, challenges in the identification of biomarkers for PCa lie primarily in the heterogeneity of the cancer itself, as well as the plasticity of the cancer genome [19]. Moreover, the predominance of disease metastatic to the bone presents its own unique problems in tissue preparation and sample acquisition.

3 Prostate-Specific Antigen

Prostate-specific antigen (PSA) has become the most widely utilized molecular marker for the diagnosis and management

of a malignancy since its identification in 1969 and its characterization in the 1980s [20–23]. The use of PSA as a PCa detection tool in asymptomatic men is controversial, primarily due to the resulting overtreatment and associated morbidities of tumors that would have otherwise remained indolent and would not have ultimately been the cause of death, and also to some extent due to declines in localized/regional PCa incidence rates [24–26]. However, distant-stage PCa rates have increased in younger men (ages 50–69) in recent years [26]. This data highlights the importance of PCa screening, especially by monitoring PSA levels after diagnosis and treatment, and its utility as a prognostic, pharmacodynamic, and surrogate marker.

A number of PSA metrics, in addition to absolute levels, have been developed and studied as markers in a variety of patient populations for just about every PCa treatment available. These metrics include PSA response, PSA doubling time, PSA velocity, PSA nadir and time-to-nadir, and PSA progression; and their utility and validation as biomarkers have been reviewed extensively [27, 28].

A "positive" PSA response to treatments for CRPC is defined as a decrease from baseline (treatment initiation) of ≥50%; this metric was used as a secondary endpoint in phase 3 trials assessing treatment efficacy for docetaxel [29, 30], abiraterone [31, 32], enzalutamide [33, 34], and cabazitaxel [35]. Despite its wide use across current trials, this PSA response of ≥50% has not been consistently validated as a surrogate marker for OS using the Prentice criteria, likely due to differences in patient populations and therapeutic agents and the heterogeneity of the disease [36–40]. Similarly, a PSA decline of ≥30% met the Prentice criteria for surrogacy when evaluated in phase 3 first-line chemotherapy [37, 38] or abiraterone (post- and prechemotherapy) [40] trials, and was partially met for enzalutamide (postchemotherapy) [36], but the criteria for surrogacy were not met in a second-line chemotherapy trial [39] (Table 1). A recent study showed that an early increase in PSA levels (≥20%, 4 weeks) was helpful in predicting progression-free survival (PFS) and OS and could help in identifying patients unlikely to benefit from enzalutamide therapy [56]. Modeling analysis of data from abiraterone phase 3 studies found a consistent treatment effect of abiraterone on PSA kinetics. Importantly, the analysis also revealed strong associations between PSA kinetics and OS in metastatic CRPC (mCRPC) patients previously treated with chemotherapy and those who were chemotherapy-naïve [40]. Recent data suggest the percentage of PSA decline 4 weeks after initiating therapy with abiraterone is predictive of PSA changes at 12 weeks and of OS [57]. PSA is not a reliable marker for measuring response to CRPC immunotherapies such as sipuleucel-T [58].

In their updated guidelines for the trial design and objectives for assessing therapies for the management of CRPC, the Prostate Cancer Working Group 3 still recommends that PSA levels be monitored [59]. The inclusion of PSA monitoring in ongoing and future CRPC clinical trials will provide additional data regarding PSA kinetics in response to various therapeutic modalities. The existing depth of data regarding PSA kinetics in response to evaluated CRPC therapies also allows the performance of other potential biomarkers to be measured against those of PSA kinetics.

PSA progression—the rise in PSA over a given threshold [60, 61] that often signals continued cancer proliferation or relapse despite treatment—usually precedes clinical or radiographic progression and death. As such, it is often the signal that treatment may no longer be effective. When evaluating individual patient responses to therapy, both PSA response and PSA progression should be used in context with the assessment of other clinical factors to guide clinical decisions.

4 Blood-Based "Liquid Biopsies"

4.1 Circulating Tumor Cells

CTCs are tumor cells found in the blood. These cells putatively originate from sites of malignancy and likely have the potential to metastasize. CTC enumeration using the CellSearch assay (Janssen Diagnostics, LLC; Raritan, NJ, USA) is the only validated, FDA-cleared biomarker to monitor patients with metastatic PCa [62]. A threshold of 5 CTCs per 7.5 mL of blood is the established cutoff point for favorable outcomes to treatment in CRPC. At initiation of therapy with either docetaxel or abiraterone, CTC counts ≤5/7.5 mL are significantly associated with better prognosis, including prolonged survival [41–43, 63, 64]. In addition, changes in CTC count during therapy are predictive of outcomes; an increase from ≤5/7.5 mL at baseline to >5/7.5 mL is associated with poorer prognosis than a change from >5 to ≤5/7.5 mL, and may have superior predictive power compared to posttreatment PSA responses of ≥30% or ≥50% [41–43, 65–67] (Table 1). Thus, a rise in CTC count during therapy is a significant predictor of poor OS and may indicate the need to switch therapy. Recently, CTC count in combination with the more classic marker lactose dehydrogenase met the Prentice criteria for surrogacy for OS at the individual-patient level in a phase 3 trial that evaluated the efficacy and safety of abiraterone in CRPC patients progressing after docetaxel therapy [44].

The CellSearch assay employs immunomagnetic capture of CD45-, anti-epithelial cell adhesion molecule (EpCAM) + cells to enrich samples for CTCs. Therefore, in patients with tumors lacking expression of EpCAM, CellSearch will not be able to detect their CTCs, and a different technique is needed [62]. The use of EpCAM-based enrichment techniques can fail to detect CTC populations that have undergone epithelial-mesenchymal transition. This may explain clinical results where low CTC numbers have been

Table 1 Summary of clinical assessment of biomarkers

Biomarker	Patient population	Treatment	Association with outcomes or validation
PSA response; ≥50% or ≥30% decline in 3 months	mCRPC	Docetaxel [38]	≥50% did not meet criteria for surrogacy for OS ≥30% met criteria for surrogacy for OS
	mCRPC	Docetaxel [37]	≥50% did not meet criteria for surrogacy for OS ≥30% met criteria for surrogacy for OS
	mCRPC, post-docetaxel and docetaxel-naïve	Abiraterone [40]	Met criteria for surrogacy for OS for both populations
	mCRPC, post-docetaxel	Enzalutamide [36]	Strongly associated with biochemical and radiologic PFS and with OS, but did not meet criteria for surrogacy for OS
	mCRPC, post-docetaxel	Cabazitaxel [39]	Statistically significant predictor of OS, but did not meet criteria for surrogacy for OS
CTC enumeration; <5 vs. ≥5 CTCs/7.5 mL	mCRPC	Any new chemotherapy [41]	CTC count <5 at baseline and at all time points assessed during treatment strongly associated with OS
	mCRPC	Docetaxel [42, 43]	Baseline count ≥5 strongly associated with worse OS
	mCRPC, post-docetaxel	Abiraterone [44]	CTC count ≥5 was a strong predictor of OS, but did not meet criteria for surrogacy CTC + lactose dehydrogenase (>250 U/L) together met criteria for surrogacy for OS
AR-V7	mCRPC, could be previously treated with taxane-based chemotherapy, abiraterone, or enzalutamide	Abiraterone or enzalutamide [45–47]	Presence of AR-V7 in CTCs was significantly associated with lower PSA response, shorter PSA PFS, clinical or radiographic PFS, and OS
	mCRPC, could be previously treated with taxane-based chemotherapy, abiraterone, or enzalutamide	Taxane-based chemotherapy [46, 48]	No significant difference observed in clinical responses to taxanes in patients with or without CTC-detected AR-V7 Patients with AR-V7 have better outcomes with taxanes than with androgen-targeting therapies
BRCA1/2 or *ATM* mutations	mCRPC, previously treated with docetaxel, abiraterone and/or enzalutamide	Olaparib [49]	Eighty-eight percent of patients with any DNA-repair gene mutations/deletions responded to treatment vs. 6% of patients without these mutations; radiologic PFS and OS were significantly longer
TMPRSS2-ERG translocation	mCRPC	Docetaxel [50, 51]	Docetaxel resistance may be twice as likely in patients with the translocation vs. those without Patients carrying the translocation had significantly lower PSA rate of PSA response and significantly worse PSA PFS, clinical/radiologic PFS, and OS
	mCRPC, post-docetaxel	Abiraterone [52]	Presence of translocation in CTCs was not predictive of response
	mCRPC, docetaxel-naïve	Abiraterone [53]	Translocation status did not significantly impact treatment efficacy
PTEN loss	mCRPC, post-docetaxel	Abiraterone [54]	PTEN loss, detected by ICH, was predictive of poorer OS
Interleukin-6	mCRPC	Docetaxel [55]	High IL-6 levels were significantly associated with reduced PFS and OS

AR-V7, androgen receptor splice variant 7; *ATM*, ataxia telangiectasia mutated; *BRCA*, breast cancer susceptibility gene; CTC, circulating tumor cell; ICH, immunohistochemical; mCRPC, metatstatic castration-resistant prostate cancer; OS, overall survival; PFS, progression-free survival; PSA, prostate-specific antigen; *PTEN*, phosphatase and tensin homolog on chromosome 10; *TMPRSS2-ERG*, transmembrane protease serine 2 (TMPRSS2)–v-ets erythroblastosis virus E26 oncogene homolog (ERG) fusion.

reported even in patients with late metastatic cancers [68]. The CellSearch system detects CTCs in ~60% of patients with CRPC [41, 65].

Other modes of CTC capture have been developed that allow for the capture of CTCs that may be missed by CellSearch and have been utilized in clinical studies to evaluate responses to therapy; these include the AdnaTest platform (Qiagen; Hilden, Germany) and the Epic Sciences platform (Epic Sciences; San Diego, CA, USA). The AdnaTest platform uses a two-step process that enriches for CTCs via immunomagnetic capture and then utilizes reverse transcriptase-based polymerase chain reaction (RT-PCR) to molecularly analyze captured CTCs. Immunomagnetic capture is carried out with antibodies for EpCAM and human growth factor receptor 2 conjugated to magnetic particles. Primers for mRNA transcripts of the genes encoding PSA, prostate-specific membrane antigen, and epidermal growth factor receptor are then employed for RT-PCR [69]. While the AdnaTest does not provide CTC enumeration, it detects CTCs in greater than 62% of CRPC patients [45, 69–71] and may provide greater sensitivity than CellSearch for CTC detection [71]. The presence of CTCs detected by the AdnaTest was associated with shorter OS in CRPC patients receiving docetaxel therapy and correlated with PSA levels [70].

The Epic Sciences CTC platform does not use cell enrichment, depletion, or microfluidic manipulation; an automated system evaluates slides of nucleated blood cells stained with a cocktail of immunofluorescent antibodies targeting cytokeratins (CK) and CD45, and 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI) [72]. Once candidate CTCs are identified, they are evaluated by trained technicians for confirmation and classification. With this platform, additional types of CTCs can be identified and included in enumeration: 1) "traditional" CTCs – CK+, CD45-, intact DAPI+ nuclei, and larger and morphologically distinct from surrounding white blood cells; 2) small CTCs – CK+, CD45-, intact DAPI+ nuclei, and similar or greater size than white blood cells; 3) CTC clusters – ≥2 CTCs, with ≥1 traditional CTC, and shared cytoplasmic boundaries; 4) CK- CTCs – CK-, CD45-, with intact DAPI+ nuclei; and 5) apoptotic CTCs – CK+ , CD45-, with a DAPI pattern of staining consistent with apoptosis [72, 73]. In samples tested from patients with mCRPC, all patients had ≥1 CTC detected per 1 mL of blood [72, 73]. In addition, CTC counts were significantly correlated with those obtained by CellSearch [73]. It should be noted that the Epic Science platform is centrally located; it is not available as a kit. All samples must be sent to the company for analyses and are stored in a repository.

In addition to enumeration of CTCs as a biomarker, captured CTCs can serve as material for the detection of other biomarkers as they are much easier to obtain than metastatic tumor tissue. CTCs identified by CellSearch, AdnaTest, and the Epic Science platform can all be further analyzed for the presence of biomarkers [45–48, 52, 73]. In the discussions below, CTCs were often the source of material for biomarker interrogation.

4.2 Circulating Tumor DNA

Circulating tumor DNA (ctDNA) or cell-free tumor DNA is released by malignant cells—either necrotic or apoptotic tumor cells or by CTCs—and can be detected as cell-free DNA in the peripheral blood of cancer patients. It is not found in peripheral blood of healthy individuals [74, 75]. ctDNA concentrations may serve as a biomarker as increased concentrations of ctDNA were significantly correlated with metastatic PCa vs. localized PCa and with the presence of CTCs in peripheral blood, suggesting a positive correlation between DNA levels and tumor stage [75]. In addition, in a small retrospective study of mCRPC patients progressing after treatment with docetaxel and then treated with abiraterone, a higher pretreatment ctDNA level was associated with shorter OS after multivariate adjustment [76]. ctDNA can also serve as source material for additional genetic biomarker analyses. While there are numerous commercial kits available for the extraction, quantification, and PCR-based molecular evaluation of ctDNA, they lack standardization and validation [74, 75].

5 Androgen Receptor

The androgen receptor (AR) plays a key role in the progression of PCa to CRPC, as is evidenced by the rise in PSA levels that signals progression despite castration levels of testosterone. One mechanism by which PCa cells "escape" the restraints of medically or surgically induced castration is through the selection for and increased expression of AR splice variants. Multiple AR splice variants that have lost their ligand-binding domain, yet remain constitutively active, have been identified in metastatic and CRPC cells [77]. AR variants have been implicated in the development of resistance to enzalutamide in in vitro studies [78, 79]. One such variant that has been investigated extensively is AR splice variant 7 (AR-V7), which is expressed in CRPC tissue samples [77].

In a hypothesis-generating study, CRPC patients treated with either enzalutamide or abiraterone acetate and carrying the AR-V7 receptor variant detected in CTCs had significantly lower PSA response rates to therapy ($p = 0.004$); significantly shorter biochemical, radiological, or clinical PFS ($p < 0.001$); and significantly shorter OS ($p \leq 0.006$) compared with those who did not carry the variant [45]. No patient expressing the AR-V7 variant experienced a PSA response (reduction of ≥50% from baseline) to treatment with abiraterone or enzalutamide. A recent larger, follow-up study confirmed

the negative prognostic impact of CTC-based AR-V7 detection in CRPC patients receiving either abiraterone or enzalutamide [47]. In contrast, AR-V7-positive patients treated with taxanes had superior ($p < 0.001$) clinical outcomes compared with those treated with abiraterone or enzalutamide [46, 48]. It should be noted, however, that among some CRPC patients carrying the AR-V7 variant, there is evidence of a PSA response to abiraterone or enzalutamide, albeit shorter than observed in those without the variant [47, 80] (Table 1). Taken together, these data suggest that patients with the AR-V7 variant may derive the most benefit from taxane-based chemotherapy, but the presence of the variant does not necessarily preclude use of abiraterone or enzalutamide. As such, the most recent NCCN guidelines state that "limited data suggest a possible role for AR-V7 testing to help guide selection of therapy" [10]. The presence of nuclear-specific AR-V7 protein may provide more fine-tuned information to guide in treatment selection, as no patient with AR-V7 protein localized to the nucleus (vs. diffusely detected in the cytoplasm) experienced a PSA response to abiraterone or enzalutamide [81]. In addition, patients with CTCs positive for nuclear-specific AR-V7 protein who received taxane-based chemotherapy experienced greater OS than those receiving androgen-targeted therapy [81]. Diffuse cytoplasmic AR-V7 staining was not predictive of response to therapy.

In addition to selection for ligand-independent AR variants, increase in *AR* copy number (gene amplification) is another means by which PCa cells "escape" low androgen levels induced by medical or surgical castration and progress to CRPC [82]. Up to 50% of men with CRPC may have substantial *AR* gene amplification when detected in CTCs by fluorescence in situ hybridization (FISH) [83]. The implications of increased *AR* copy number or expression in response to treatment with abiraterone or enzalutamide has not been extensively studied. One small study found significant increases in *AR* expression in patients who were progressing after abiraterone ($n = 10$) or abiraterone followed by enzalutamide ($n = 6$) compared with patients who were abiraterone-naïve ($n = 10$) [84], while in another small study, no shift in *AR* copy number was observed with treatment with abiraterone ($n = 18$) [85]. Interrogations of ctDNA from mCRPC patients for *AR* copy number prior to treatment with enzalutamide or abiraterone found that pretreatment *AR* copy number gain was significantly associated with shorter PFS and OS for either therapy [76, 86, 87].

6 Mutations in DNA Repair Genes; Focus on *BRCA2*

The well-recognized heterogeneity of advanced PCa is partially attributed to altered proteins involved in various DNA repair pathways; the role of mutated DNA repair genes in PCa has been recently reviewed [88]. Roughly 20–30% of mCRPC cases have mutations in DNA repair genes; a proportion of which are germline [88–90]. The prevalence of germline mutations in men with metastatic PCa is significantly greater than that in men with localized disease or in the general population [90].

The breast cancer susceptibility gene 2 (*BRCA2*) codes for an important protein in the pathway responsible for repairing double-strand DNA breaks [88] and was first associated with familial breast and ovarian cancer. In PCa, overall, *BRCA2* mutations account for the 1.2% to 1.8% of cases, and the presence of a *BRCA2* germline mutation in men with newly diagnosed PCa is associated with more aggressive disease and poor survival outcomes [91]. Genomic analysis of genes involved in DNA repair in 692 men with metastatic PCa detected pathogenic germline mutations in 16 different DNA repair genes in 82 (11.8%) men; the largest proportion, 44%, were in *BRCA2*. In contrast, among 499 men with localized PCa, 23 (4.6%) had germline mutations for DNA repair genes ($p < 0.001$ vs. metastatic PCa) [90].

Poly(adenosine diphosphate-ribose) polymerase (PARP) inhibitors interfere with cellular ability to repair certain types of DNA damage; when used to treat cancers with certain DNA repair gene defects, a synergistic lethal effect occurs [88]. One such PARP inhibitor, olaparib, approved for use in ovarian cancer, was assessed for efficacy in men with mCRPC previously treated with docetaxel, abiraterone, or enzalutamide, and/or cabazitaxel. Tissue samples were retrospectively evaluated for the mutational status of a panel of DNA repair genes including *BRCA1*, *BRCA2*, and ataxia telangiectasia mutated (*ATM*) and correlated with outcome [49]. Initial results from 49 patients revealed that men positive for gene panel mutations had a significantly higher response rate to olaparib ($p < 0.001$); 14/16 had a response. Radiographic PFS was significantly longer and OS was prolonged in patients positive for mutations in the DNA gene panel compared with those negative for these mutations. All 7 patients with biallelic loss of BRCA2 function (3 germline mutation carriers) responded to treatment with PSA declines of ≥50% from baseline [49] (Table 1). This study (NCT01682772) is ongoing. Based on these results, olaparib received breakthrough therapy designation from the FDA in January 2016 for the treatment of mCRPC in patients with *BRCA1/2* or *ATM* mutations who had received prior taxane-based chemotherapy and abiraterone or enzalutamide [92]. Additional studies are under way evaluating olaparib as monotherapy or in combination with other agents for the treatment of mCRPC (NCT01972217, NCT02893917, NCT02987543, NCT03012321). Thus, if olaparib is approved by the FDA for wide use in men with *BRCA1/2* or *ATM* mutations, screening men at initial diagnosis may be indicated.

7 TMPRSS2-ERG Translocation

The transmembrane protease serine 2 (*TMPRSS2*)–v-ets erythroblastosis virus E26 oncogene homolog (*ERG*) fusion

is created by the translocation of the androgen-driven 5′ *TMRPSS2* chromosomal region to the E-twenty six (*ETS*) transcription factor family member ERG on chromosome 21q22.2 [93]. This translocation is present in approximately 50% of localized PCa [93]. The clinicopathologic impact of this fusion is not clear; however, it is known that it creates AR-driven expression of *ERG* and can be used to monitor AR activity. *TMPRSS2-ERG* fusion status displays low intrapatient variability and these rearrangements tend to be consistent across samples obtained at various stages of PCa development and progression, suggesting it occurs early in PCa development [85]. The *TMPRSS2-ERG* fusion gene is expressed in CRPC at levels comparable to those in untreated primary PCa, presumably reflecting reactivation of AR [94]. Because of this, the presence of the fusion has been investigated as a biomarker [85].

There are two types of *TMPRSS2-ERG* fusion rearrangements, which are usually detected using FISH: the Edel (deletion) and Esplit (insertion) [93]. The Edel subtype of *TMPRSS2-ERG* fusion represents an aggressive molecular subtype susceptible to higher recurrence, which is more likely to evolve to progress to metastasis and CRPC [95, 96]. Overexpression of ERG in in vitro and in vivo models of CRPC leads to taxane resistance by interfering with the ability of docetaxel or cabazitaxel to engage tubulin [50]. Men with mCRPC and *TMPRSS2-ERG* rearrangement are twice as likely to develop resistance to docetaxel [50]. Indeed, detection of *TMPRSS2-ERG* in blood was predictive of resistance to both docetaxel and cabazitaxel in men with mCRPC and, importantly, among patients negative for the rearrangement at baseline and progressed on taxane treatment, 41% became positive for the rearrangement at progression [97] (Table 1).

In a phase 2 abiraterone study in men with progressing CRPC after chemotherapy, the *TMPRSS2-ERG* fusion did not predict response to abiraterone therapy [52]. However, in a secondary analysis of a phase 3 study evaluating the efficacy of abiraterone in chemotherapy-naïve men with CRPC, among patients in whom ERG status could be determined (n = 348), those with 2 + Edel (2 or more FISH-detected *TMPRSS2-ERG* Edel rearrangements per cell), which is associated with worse outcomes, derived significantly greater benefit from therapy, with improved radiographic PFS and longer time to PSA progression [53]. This would suggest that men newly progressed to CRPC carrying the *TMPRSS2-ERG* Edel rearrangement may be more likely benefit from treatment with abiraterone prior to docetaxel.

8 PTEN Loss

Phosphatase and tensin homolog on chromosome 10 (*PTEN*) is one of the most frequently inactivated tumor suppressor genes in PCa and loss of *PTEN* is associated with aggressive disease [98]. Along with its role in suppressing the activation of the serine-threonine kinase Akt, *PTEN* directly interacts with AR, preventing AR translocation from the cytosol to the nucleus, promoting AR protein degradation, and inhibiting AR transactivation [99]. Thus, *PTEN* loss may be a useful prognostic biomarker in PCa [98, 100].

PTEN status can be evaluated via FISH or immunohistochemical (IHC) protein staining. FISH will miss small deletions or mutations that knock out *PTEN* expression; thus, IHC staining may be more sensitive in detecting *PTEN* loss [51, 101]. A number studies have demonstrated the heterogeneity of *PTEN* loss in PCa when FISH was used to detect loss, wherein IHC assays were able to efficiently detect *PTEN* loss [98, 100]. These reports suggest that, compared with FISH analysis, IHC assays may be more accurate in detecting *PTEN* loss and, therefore, may be useful for stratifying risk in PCa.

Among the 144 mCRPC patients who were progressing after docetaxel therapy and then received abiraterone for which both hormone-naïve PCa and CRPC tissue samples were available for IHC evaluation of *PTEN* status, *PTEN* loss was detected in 38% of primary tumor samples and in 50% of mCRPC samples; intrapatient concordance was 90% [54]. In 41 patients for whom both hormone-sensitive and CRPC tissue were available, *PTEN* status was concordant in 86% of cases. In multivariate analysis, loss of *PTEN* among these postchemotherapy mCRPC patients receiving abiraterone was associated with decreased duration of treatment (HR 1.6; 95% CI, 1.12–2.28; p = 0.009) and reduced OS (HR 1.75; 95% CI 1.19–2.55; p = 0.004) [54] (Table 1). Thus, *PTEN* loss may serve as a predictive marker for response to abiraterone treatment, likely as part of a wider panel of predictive biomarkers. Additional prospective, larger studies are needed to confirm these results.

9 Interleukin-6

Serum levels of interleukin-6 (IL-6), a marker of chronic inflammation in cancer, are often elevated in metastatic PCa and CRPC [102–105]. In vitro data suggest that elevated levels of IL-6 may work synergistically with *PTEN* loss to promote PCa progression [106], may increase levels of intracrine androgen production through the up-regulation of the expression of enzymes involved in tumoral androgen synthesis [107], and confer drug resistance, both to cytotoxic and androgen deprivation therapies, via anti-apoptotic mechanisms [108, 109].

Higher baseline IL-6 is associated with poor prognosis in CRPC [103, 110]. In a phase 3 study of patients with mCRPC that assessed the efficacy and safety of suramin, IL-6 levels above the study population-defined median were significantly associated with worse survival compared with IL-6 levels at or below the median [110]. A later study of patients with mCRPC receiving first-line docetaxel prospectively tested

for IL-6 before and after chemotherapy [55]. In a multivariate analysis, pretreatment IL-6 level was the only independent prognostic factor for time-to-PSA progression and was the only independent predictor for survival, while other variables were not. Moreover, analysis of IL-6 changes under therapy also suggested a correlation between a PSA response and a decrease of IL-6 [55]. Similar results were observed by Ignatoski, et al.; among patients with mCRPC receiving first-line docetaxel therapy, there was a mean 35% decrease in IL-6 levels among those with a PSA response compared with a mean 76% increase among nonresponders ($p = 0.03$) [111]. These data suggest that IL-6 may serve as both a predictive and pharmacodynamic marker to gauge response to therapy.

Considering its increased levels in more advanced PCa and its potential role in PCa progression, IL-6 has also been investigated as a therapeutic target. In a phase 1 study, men with localized PCa undergoing radical prostatectomy were treated with siltuximab, a monoclonal anti-IL-6 antibody, or placebo. Treatment with siltuximab led to down-regulation of genes immediately downstream of the IL-6 signaling pathway and key enzymes of the androgen signaling pathway [112]. However, in later phase 2 studies, siltuximab did not confer additional benefit to patients with mCRPC when used as monotherapy or when coadministered with mitoxantrone + prednisone vs. mitoxantrone + prednisone alone, although it was well tolerated [113, 114]. In a more recent phase 1 study in patients with mCRPC, siltuximab in combination with docetaxel demonstrated preliminary efficacy [115]. Additional phase 2 and 3 studies in various PCa patient populations are needed to determine if siltuximab alone or in combination with newer cytotoxic and hormonal therapies (abiraterone acetate and enzalutamide) in the background of elevated IL-6 levels would be of benefit to patients with advanced PCa.

10 Conclusions

Management of CRPC has come a long way in the past decade, yet the complexity of the disease lends itself to varying responses to therapies in different patients. In addition to what has been reviewed here, many other molecules and molecular techniques have been identified as potential biomarkers for PCa, too many to include here. These include microRNAs [116, 117], markers of epithelial-to-mesenchymal transition [116, 118, 119], markers of bone turnover [120, 121], and the use of imaging and specialized imaging tracers [122–124]. Furthermore, other sources for biomarker detection are under investigation, particularly exosomes [125].

While biomarkers such as PSA and CTCs provide information regarding treatment response, no validated biomarkers have been adapted to aide in determination of optimal treatment sequencing, and surrogacy endpoints are limited. Identification, characterization, and validation of biomarkers that can serve as surrogates for clinical outcomes traditionally used as endpoints in clinical trials, such as PFS and OS, will allow for more rapid evaluation of emerging therapies. Furthermore, as additional therapies emerge for the management of CRPC, the identification and utilization of multiple biomarkers that will aid in optimal personalized management and treatment sequencing leading to prolonged survival becomes even more important.

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Compliance with Ethical Standards

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