

Prognostic and predictive biomarkers in prostate cancer: latest evidence and clinical implications

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Abstract: Advances in our understanding of the mechanisms driving castration-resistant prostate cancer have promoted the development of several new drugs including androgen receptor-directed therapy and chemotherapy. Concomitant docetaxel treatment at the beginning of hormonal therapy for metastatic prostate cancer has resulted in longer overall survival than with hormonal therapy alone. Elucidating an appropriate treatment sequence using these therapies is important for maximizing clinical benefit in castration-sensitive and castration-resistant prostate cancer patients. The development of advanced high-throughput ‘omics’ technology has enabled the use of novel markers to guide prognosis and treatment of this disease. In this review, we outline the genomic landscape of prostate cancer and the molecular mechanisms of castration-resistant progression, and how these affect the development of new drugs, and their clinical implications for selecting treatment sequence. We also discuss many of the potential tissue-based or liquid biomarkers that may soon enter clinical use, with the hope that several of these prognostic or predictive markers will guide precision medicine for prostate cancer patients in the near future.

Keywords: prostate cancer, biomarker, treatment, castration-resistant prostate cancer

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Introduction

Prostate cancer is the second leading cause of cancer death in the USA¹ and the number of cases are rapidly increasing in Japan.² Patients presenting with advanced disease typically receive hormonal therapy using medical or surgical castration as initial treatment. However, most prostate cancer patients acquire resistance to the initial hormonal therapy over 2–3 years, thus progressing to a castration-resistant disease state.³

Since docetaxel was introduced in 2004 to prolong the survival of patients with castration-resistant prostate cancer (CRPC),⁴ there has been a rapid increase in the number of effective systemic agents for CRPC, including novel androgen receptor (AR)-directed, immunotherapeutic, chemotherapeutic and radiopharmaceutical drugs. Concomitant docetaxel treatment at the beginning of hormonal therapy for metastatic

castration-sensitive prostate cancer (CSPC) has resulted in longer overall survival than with hormonal therapy alone.⁵ Elucidating an appropriate treatment sequence is important for maximizing clinical benefit in CSPC and CRPC patients. Improvements in technology aimed at genomic, transcriptomic and metabolomic analysis have led to the discovery of an abundance of new biomarkers that may be utilized in the prediction of prostate cancer outcome and response to therapy.⁶ The characterization of tumor tissue through advanced high-throughput ‘omics’ technology may subsequently create personalized road maps to guide clinical decision-making because of better understanding of the patient’s risk of progression.⁷ Here, we summarize the utilization of prostate cancer biomarkers in current clinical practice (Table 1), their advantages and limitations, and possible future considerations for their use to guide therapy.

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Table 1. Potential prognostic or predictive biomarkers in prostate cancer.

Biomarker	Source	Clinical relevance	Prog versus Pred
Metastatic status	Clinical	Number of bone mets (EOD), visceral mets	Prog/Pred
Performance status	Clinical	ECOG performance status (0–4)	Prog/Pred
Time to CRPC	Clinical	Time from ADT to CRPC	Pred
Prior treatment	Clinical	Number of antiandrogens or steroid	Pred
PSA	Blood	Protein specifically extracted from prostate gland	Prog
PSA kinetics	Blood	PSA decrease rate under treatment	Prog
Gleason score	Tissue	Pathological features strongly correlated prognosis	Prog/Pred
Lactate dehydrogenase	Blood	Elevated by injuries and various disease including cancer	Prog/Pred
Alkaline phosphatase	Blood	Elevated by cancer spreading to bones or liver	Prog
Albumin	Blood	An index of nutritional status	Prog
Hemoglobin	Blood	Decreased by anemia	Prog/Pred
Neutrophil-lymphocyte ratio (NLR)	Blood	Elevated NLR predicted poorer OS in various cancer patients	Prog
Testosterone	Blood	Ligand of AR associating prostate cancer proliferation	Prog/Pred
Number of circulating tumor cells (CTCs)	Blood	Increased number of CTCs associating with worse cancer prognosis	Prog
AR splice variants in CTC (esp. AR-V7)	Blood	Correlating with poor response to ENZA and ABI but good response to Chemo	Pred
Concentration of cell-free DNA (cfDNA)	Blood	Increased abundance of cfDNA associating with worse cancer prognosis	Prog
AR mutation and copy number in cfDNA	Blood	Correlating with worse efficacy of ENZA and ABI	Pred
Somatic DNA repair mutations	Tissue	Correlating with poor response to ADT, but good response to PARP inhibitors	Prog/Pred

ABI, abiraterone; ADT, androgen-deprivation therapy; AR, androgen receptor; cfDNA, cell-free DNA; CRPC, castration-resistant prostate cancer; CTC, circulating tumor cell; ECOG, Eastern Cooperative Oncology Group; EOD, extent of disease; ENZA, enzalutamide; mets, metastases; NLR, neutrophil-lymphocyte ratio; OS, overall survival; PARP, poly-ADP ribose polymerase; Pred, predictive marker, Prog, prognostic marker; PSA, prostate-specific antigen.

Pretreatment clinical parameters as prognostic or predictive biomarkers

The currently used biomarkers are defined as prognostic and predictive (Table 1). Prognostic markers aim to evaluate objectively the patient's overall outcome, such as the probability of cancer recurrence after standard treatment. The presence or absence of a prognostic marker can be useful for the selection of patients for treatment but does not directly predict the response to treatment. Predictive markers aim to evaluate objectively the likelihood of benefit from a specific clinical intervention, or the differential outcomes of two or more interventions, including toxicity.⁸

The discovery of prostate-specific antigen (PSA) as a serum tumor marker has revolutionized prostate cancer diagnosis, and is the only widely used biomarker for diagnosis and prognosis of this disease. However, PSA is organ- but not cancer-specific. Moreover, it is not able to differentiate between indolent and aggressive forms of prostate cancer. Many men may harbor aggressive prostate cancer despite having low initial levels of serum PSA.⁹ The Gleason grading system is also used with prostate biopsy samples to help evaluate the prognosis of men with prostate cancer.¹⁰ Recently, the new, simplified prostate cancer grading system with five grades has shown more accurate grade stratification

compared with current Gleason grading systems.¹¹ Together with other parameters, it is incorporated into a strategy of prostate cancer staging that predicts prognosis and helps to guide treatment.

The majority of other clinical and biological prognostic biomarkers of prostate cancer have been validated, which helps physicians to estimate survival using tumor and patient characteristics. Originally, PSA-based test results, performance status (PS) score and hemoglobin level were combined with age, albumin, lactate dehydrogenase (LDH) or alkaline phosphatase levels, Gleason score, pain intensity and metastases characteristics in different prognostic models.^{12,13} After the emergence of new treatment options in CRPC, an updated nomogram for predicting survival in men with metastatic CRPC receiving first-line chemotherapy was developed and validated.¹⁴ More recently, metastatic site and opioid analgesic use,¹⁵ as well as serum androgen levels,¹⁶ have been reported to correlate with prognosis. We analyzed pretreatment parameters predicting enzalutamide efficacy by Cox proportional hazard analyses for PSA progression-free survival in 345 patients. Enzalutamide treatment was effective for patients with low Gleason scores, good PS, absence of bone or visceral metastasis and no prior steroid or docetaxel treatment.¹⁷ In subgroup analysis of the PREVAIL study, the treatment effect of enzalutamide for increasing progression-free survival time was more significant in patients with good PS, low Gleason score, no visceral metastasis, low LDH levels and high hemoglobin levels.¹⁸ For metastatic CSPC, the benefit of chemohormonal therapy was more apparent in the subgroup with high-volume than low-volume disease, indicating that the clinical benefit was more pronounced among patients with a higher burden of disease.⁵ The neutrophil-to-lymphocyte ratio is also correlated with prognosis in patients with metastatic prostate cancer.¹⁹ By using these known baseline clinical parameters, we can predict the prognosis and efficacy of novel treatments for prostate cancer. However, they are not perfect for selecting the best treatment sequence. Achieving precision medicine will require more precise tissue- or liquid-based biomarkers with prognostic and predictive value beyond these clinical parameters.

Treatment selection based on the mechanisms of castration resistance

Most advanced prostate cancers treated with androgen-deprivation therapy (ADT) acquire

castration resistance by various mechanisms, including AR overexpression, AR mutation, AR activation by other signals and non-AR pathways.²⁰ Novel mechanisms such as *de novo* androgen production in cancer cells²¹ and the generation of AR splice variants²² have recently been associated with castration resistance and poor prognosis. We have previously reported that a prostate cancer cell line, LNCaP, comprises a heterogeneous group of cells with different androgen-deprivation sensitivities and potential for invasiveness.²³ Therefore, we need to consider the heterogeneity of CRPC cells when we choose therapy for each prostate cancer patient.

The mechanisms of castration resistance and the treatment selection based on them are summarized in Table 2. AR overexpression was associated with castration resistance in a study using mouse xenograft models of prostate cancer, and enzalutamide suppressed tumor growth,²⁴ indicating that enzalutamide is effective for CRPC patients with increased AR expression. AR mutations might also be induced by ADT or specific antiandrogens.^{25–27} For patients with CRPC harboring mutant AR, antiandrogen withdrawal or alternative antiandrogen treatment might be effective.²⁸ Enzalutamide-refractory mutant AR has been reported recently and might be one mechanism for acquired enzalutamide resistance.²⁹ The testosterone concentration of metastatic prostate cancer tissues is higher than in nonmetastatic tissues, caused by the increased expression of enzymes for androgen synthesis such as cytochrome P450 17 α -hydroxylase/17,20-lyase (CYP17).²¹ Abiraterone might be effective for these CRPC patients. Recently, the metabolites of abiraterone have been demonstrated to have antagonistic effects on AR and considered to have a further potential mechanism of action.³⁰ AR splice variants including AR-V7 have been shown to provide an important mechanism for CRPC and treatment resistance.³¹ They are AR isoforms coding only for the DNA binding and transactivation domains of AR, and lack the C-terminal ligand-binding domain.³² These truncated AR species are resistant to conventional AR-targeting agents as well as abiraterone and enzalutamide.^{33,34} Taxane chemotherapy might be a better treatment option for AR-V7-positive prostate cancer patients.^{35–37} Therefore, detection of AR-V7 might represent a prognostic and predictive (i.e. treatment selection) marker in men with CRPC.³⁸ Other than AR overexpression, mutation and splice variants,

Table 2. Mechanisms of castration resistance and the treatment selections.

Mechanism	Treatment
AR mutation	Alternative antiandrogen (e.g. bicalutamide→flutamide)
AR overexpression	Novel antiandrogen (enzalutamide)
Novel androgen synthesis	CYP17 inhibitor (abiraterone)
AR splice variant	Taxane chemotherapy (docetaxel/cabazitaxel)
AR activation by other signals	Steroid/estrogen/molecular target therapy
Non-AR pathways	Chemotherapy (platinum)/molecular target therapy (PARP inhibitor)

AR, androgen receptor; CYP17, cytochrome P450 17 α -hydroxylase/17,20-lyase; PARP, poly-ADP ribose polymerase.

various additional AR bypass pathways are associated with androgen-independent AR activation and might represent future treatment options for CRPC.³⁹ We previously reported the association of a prostaglandin receptor, EP4, in CRPC as a potential treatment target.⁴⁰ Historically, steroid⁴¹ and estrogen⁴² treatment has resulted in subjective and objective responses in patients with CRPC. Glucocorticoid receptor (GR) and progesterone receptor (PR) are considered to be AR bypass pathways associated with castration resistance.⁴³ The actions of these receptors under treatment with steroids or estrogen differ among cell types and their concentrations.⁴⁴ Recently, GR overexpression was reported to be associated with enzalutamide resistance.⁴⁵ The interaction of GR or PR and AR is complicated and elucidation of their true function and clinical implication in CRPC needs further examination.⁴⁶ Non-AR pathways such as neuroendocrine differentiation⁴⁷ and DNA repair⁴⁸ are also important. As androgen-targeting therapy is not effective in these cells, platinum-based chemotherapy or other molecular targeted therapy such as poly-ADP ribose polymerase (PARP) inhibitor may be treatment options.

Circulating tumor cells and cell-free DNA as novel liquid biomarkers

Prostate biopsies are almost always performed at the time of prostate cancer diagnosis, therefore, tissue markers such as Gleason grade are useful to determine prognosis and select first-line treatment. However, for patients who fail first-line therapy, treatment is often changed without performing another tumor biopsy. Therefore, other biomarkers using samples easily obtained for predicting subsequent treatment efficacy are urgently needed. Recent advances in high-throughput

technology provide new and powerful platforms to find novel biomarkers from body fluids such as blood or urine.⁴⁹

We have previously examined the gene expression profiles using transcriptome analyses of prostate cancer tissues and found that cysteine-rich angiogenic inducer (Cyr)61 is highly expressed in prostate cancer, and its expression is correlated with cancer aggressiveness.⁵⁰ Serum Cyr61 protein expression levels are correlated with biochemical recurrence after surgery.⁵¹ Proteomic analyses have recently uncovered several candidate biomarkers from tissue or serum samples.⁵² We have examined the lipid expression profiles of prostate cancer tissues using high-resolution imaging mass spectrometry⁵³ and found that decreased expression of lysophosphatidylcholine independently predicts biochemical recurrence.⁵⁴ We have also found, using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, that a C-terminal PSA fragment composed of 19 amino acid residues is a potential novel urine biomarker for diagnosis of prostate cancer.⁵⁵ However, it was difficult to establish the method to measure the concentration of these lipids or proteins. Therefore, none of them could be commercially available biomarkers.

The blood of some patients with advanced prostate cancer contains circulating tumor cells (CTCs) derived from the primary tumor and metastatic sites. It is also known that CTCs can be detected in peripheral blood before the occurrence of clinically detectable metastases. Several CTC isolation methods have been investigated.⁵⁶ The most extensively investigated target in the context of CTC characterization in prostate cancer is the AR. We reported that the detection of AR-V7 in CTCs was associated with resistance to

abiraterone and enzalutamide.³³ AR-V7-positive CTCs were identified in 39% of patients receiving enzalutamide and 19% of those receiving abiraterone in our initial study of 62 patients. The PSA response rate was 0% for AR-V7-positive patients in the context of both therapies. These results have now been expanded to a larger sample of 202 patients, in whom the negative prognostic impact of CTC-specific AR-V7 detection has been confirmed.⁵⁷ These results suggest that the presence of AR-V7 might explain the mechanism of primary resistance to abiraterone and enzalutamide in many cases. By contrast, the presence of AR-V7 does not appear to correlate with poor treatment responses in patients receiving docetaxel or cabazitaxel.^{35,37,58}

One of the shortcomings of CTC analysis is difficulty in cell isolation and subsequent nucleic acid extraction (blood samples need immediate preparation soon after extraction from patients). In addition, CTC capture methods based on epithelial cell adhesion molecule or other cell-surface markers may miss mesenchymal cells undergoing epithelial–mesenchymal transition. Cell-free DNA (cfDNA) has recently been recognized as a potential biomarker in advanced tumors. cfDNA is composed of small fragments of nucleic acid that are not associated with cells or cell fragments.^{59,60} cfDNA might be more stable than CTCs and can be stored for several days after extraction. In several solid malignancies, analysis of cfDNA has been used to characterize and monitor disease, as well as predict outcome and treatment response.⁶¹ Similar to CTCs, ARs represent an important target in the context of cfDNA analysis. AR copy number variations and activating mutations in the ligand-binding domain are correlated with resistance to abiraterone and enzalutamide.^{26,27} In addition, analysis of AR-V7 from whole-blood RNA is feasible^{62,63} and may correlate with inferior outcomes to abiraterone and enzalutamide.⁶⁴

Novel molecular biomarkers based on genomic landscape of prostate cancer

ARs are the most important molecules for prostate cancer progression, and their overexpression, mutation or splice variance can be useful predictive biomarkers. However, other genomic changes in prostate cancer might also be useful in the recent advances of CTC and cfDNA isolation technology.

Gene fusions, specifically E26 transformation-specific fusions such as the *TMPRSS2:ERG*

translocation, are associated with early onset of prostate cancer.⁶⁵ *TMPRSS2:ERG* gene fusion might predict cancer-specific and overall survival based on immunohistochemistry in metastatic patients undergoing palliative transurethral resection of the prostate.⁶⁶ However, there seems to be no association between *TMPRSS2:ERG* expression and response to ADT.^{67,68} Phosphatase and tensin homolog (PTEN) loss activates PI3K/AKT signaling, thus controlling cell proliferation and growth⁶⁹ and is associated with poor prognosis.⁷⁰ The prognostic value of PTEN deletion combined with *TMPRSS2:ERG* fusion in prostate cancer has been investigated in several studies.^{71–73} PTEN loss is independently associated with increased risk of lethal progression, particularly in the *ERG* fusion-negative subgroup.⁷⁴ It is also reported that PTEN-negative tumors are associated with worse survival and shorter time on abiraterone treatment in CRPC.¹⁵ *TMPRSS2-ERG* fusions and PTEN gene in CTCs have been evaluated,⁷⁵ but the results are not consistent.⁷⁶ After these initial publications, several more recent reports fail to show the value of these parameters as potential predictive biomarkers. Other prostate-cancer-associated gene mutations such *MYC*, *RB1* and *MET* have also been detected in cfDNA; genomic aberrations in these genes are associated with poor prognosis.⁷⁷

It is increasingly recognized that mutations in genes controlling DNA repair pathways, especially homologous recombination repair and mismatch repair, may be relevant in many cancer types including prostate cancer.⁷⁸ In recent genomic sequencing efforts, the prevalence of somatic DNA repair gene mutations (primarily involving the *BRCA1/2* and *ATM* genes) in biopsies from patients with CRPC is in the order of 15–25%.⁷⁹ About half of these patients with somatic DNA repair aberrations also have germline defects in these same DNA repair genes (8–12% of the total).⁸⁰ The presence of a germline or somatic mutation in a DNA repair gene may have prognostic and therapeutic implications. For example, one study has suggested that these patients have poorer responses to ADT.^{81,82} Conversely, such patients may have a favorable response to alternative therapies including PARP inhibitors such as olaparib.⁴⁸ Intriguingly, patients with tumors that harbor DNA repair defects may exhibit higher sensitivity to platinum-containing chemotherapy,⁸³ immune checkpoint inhibitors,⁸⁴ radiopharmaceutical products⁸⁵ or a novel approach involving high-dose testosterone treatment.⁸⁶ In

the next few years, several ongoing studies will conclusively determine the predictive impact of DNA repair mutations in the context of these and other therapies.

Future perspective

There have been rapid advancements in the treatment of CRPC, with a resulting improvement in prognosis of patients. Further research is needed with respect to selection and sequencing of therapy^{87,88} to determine the optimal series of treatments for an individual patient. A role for biomarkers to select patients that may benefit from a particular therapy will need to be elucidated further, but the detection of the AR-V7 splice variant and DNA repair mutations appear promising candidates in the quest for biomarkers that will allow the precision medicine revolution to take place. The future of precision oncology is upon us.

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References

1. Siegel R, Ma J, Zou Z, *et al.* Cancer statistics, 2014. *CA Cancer J Clin* 2014; 64: 9–29.
2. Ito K. Prostate cancer in Asian men. *Nat Rev Urol* 2014; 11: 197–212.
3. Harris WP, Mostaghel EA, Nelson PS, *et al.* Androgen deprivation therapy: progress in understanding mechanisms of resistance and optimizing androgen depletion. *Nat Clin Pract Urol* 2009; 6: 76–85.
4. Tannock IF, de Wit R, Berry WR, *et al.* Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *N Engl J Med* 2004; 351: 1502–1512.
5. Sweeney CJ, Chen YH, Carducci M, *et al.* Chemohormonal therapy in metastatic hormone-sensitive prostate cancer. *N Engl J Med* 2015; 373: 737–746.
6. Barbieri CE, Bangma CH, Bjartell A, *et al.* The mutational landscape of prostate cancer. *Eur Urol* 2013; 64: 567–576.
7. Jeronimo C, Bastian PJ, Bjartell A, *et al.* Epigenetics in prostate cancer: biologic and clinical relevance. *Eur Urol* 2011; 60: 753–766.
8. Mehta S, Shelling A, Muthukaruppan A, *et al.* Predictive and prognostic molecular markers for cancer medicine. *Ther Adv Med Oncol* 2010; 2: 125–148.
9. Thompson IM, Pauler DK, Goodman PJ, *et al.* Prevalence of prostate cancer among men with a prostate-specific antigen level < or =4.0 ng per milliliter. *N Engl J Med* 2004; 350: 2239–2246.
10. Epstein JI. An update of the Gleason grading system. *J Urol* 2010; 183: 433–440.
11. Epstein JI, Zelefsky MJ, Sjoberg DD, *et al.* A contemporary prostate cancer grading system: a validated alternative to the Gleason Score. *Eur Urol* 2016; 69: 428–435.
12. Smaletz O, Scher HI, Small EJ, *et al.* Nomogram for overall survival of patients with progressive metastatic prostate cancer after castration. *J Clin Oncol* 2002; 20: 3972–3982.
13. Halabi S, Small EJ, Kantoff PW, *et al.* Prognostic model for predicting survival in men with hormone-refractory metastatic prostate cancer. *J Clin Oncol* 2003; 21: 1232–1237.
14. Halabi S, Lin CY, Kelly WK, *et al.* Updated prognostic model for predicting overall survival in first-line chemotherapy for patients with metastatic castration-resistant prostate cancer. *J Clin Oncol* 2014; 32: 671–677.
15. Ferraldeschi R, Nava Rodrigues D, Riisnaes R, *et al.* PTEN protein loss and clinical outcome from castration-resistant prostate cancer treated with abiraterone acetate. *Eur Urol* 2015; 67: 795–802.
16. Ryan CJ, Molina A, Li J, *et al.* Serum androgens as prognostic biomarkers in castration-resistant prostate cancer: results from an analysis of a randomized phase III trial. *J Clin Oncol* 2013; 31: 2791–2798.
17. Terada N, Akamatsu S, Okada Y, *et al.* Factors predicting efficacy and adverse effects of enzalutamide in Japanese patients with castration-resistant prostate cancer: results of retrospective multi-institutional study. *Int J Clin Oncol* 2016; 21: 1155–1161.
18. Beer TM, Armstrong AJ, Rathkopf DE, *et al.* Enzalutamide in metastatic prostate cancer before chemotherapy. *N Engl J Med* 2014; 371: 424–433.

19. Kawahara T, Yokomizo Y, Ito Y, *et al.* Pretreatment neutrophil-to-lymphocyte ratio predicts the prognosis in patients with metastatic prostate cancer. *BMC Cancer* 2016; 16: 111.
20. Feldman BJ and Feldman D. The development of androgen-independent prostate cancer. *Nat Rev Cancer* 2001; 1: 34–45.
21. Montgomery RB, Mostaghel EA, Vessella R, *et al.* Maintenance of intratumoral androgens in metastatic prostate cancer: a mechanism for castration-resistant tumor growth. *Cancer Res* 2008; 68: 4447–4454.
22. Guo Z, Yang X, Sun F, *et al.* A novel androgen receptor splice variant is up-regulated during prostate cancer progression and promotes androgen depletion-resistant growth. *Cancer Res* 2009; 69: 2305–2313.
23. Terada N, Shiraishi T, Zeng Y, *et al.* Correlation of Sprouty1 and Jagged1 with aggressive prostate cancer cells with different sensitivities to androgen deprivation. *J Cell Biochem* 2014; 115: 1505–1515.
24. Chen CD, Welsbie DS, Tran C, *et al.* Molecular determinants of resistance to antiandrogen therapy. *Nat Med* 2004; 10: 33–39.
25. Yoshida T, Kinoshita H, Segawa T, *et al.* Antiandrogen bicalutamide promotes tumor growth in a novel androgen-dependent prostate cancer xenograft model derived from a bicalutamide-treated patient. *Cancer Res* 2005; 65: 9611–9616.
26. Romanel A, Gasi Tandefelt D, Conteduca V, *et al.* Plasma AR and abiraterone-resistant prostate cancer. *Sci Transl Med* 2015; 7: 312re10.
27. Azad AA, Volik SV, Wyatt AW, *et al.* Androgen receptor gene aberrations in circulating cell-free DNA: biomarkers of therapeutic resistance in castration-resistant prostate cancer. *Clin Cancer Res* 2015; 21: 2315–2324.
28. Terada N, Shimizu Y, Yoshida T, *et al.* Antiandrogen withdrawal syndrome and alternative antiandrogen therapy associated with the W741C mutant androgen receptor in a novel prostate cancer xenograft. *Prostate* 2010; 70: 252–261.
29. Korpál M, Korn JM, Gao X, *et al.* An F876L mutation in androgen receptor confers genetic and phenotypic resistance to MDV3100 (enzalutamide). *Cancer Discov* 2013; 3: 1030–1043.
30. Li Z, Alyamani M, Li J, *et al.* Redirecting abiraterone metabolism to fine-tune prostate cancer anti-androgen therapy. *Nature* 2016; 533: 547–551.
31. Hu R, Dunn TA, Wei S, *et al.* Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. *Cancer Res* 2009; 69: 16–22.
32. Antonarakis ES, Armstrong AJ, Dehm SM, *et al.* Androgen receptor variant-driven prostate cancer: clinical implications and therapeutic targeting. *Prostate Cancer Prostatic Dis* 2016; 19: 231–241.
33. Antonarakis ES, Lu C, Wang H, *et al.* AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *N Engl J Med* 2014; 371: 1028–1038.
34. Nakazawa M, Antonarakis ES and Luo J. Androgen receptor splice variants in the era of enzalutamide and abiraterone. *Horm Cancer* 2014; 5: 265–273.
35. Antonarakis ES, Lu C, Luber B, *et al.* Androgen receptor splice variant 7 and efficacy of taxane chemotherapy in patients with metastatic castration-resistant prostate cancer. *JAMA Oncol* 2015; 1: 582–591.
36. Nakazawa M, Lu C, Chen Y, *et al.* Serial blood-based analysis of AR-V7 in men with advanced prostate cancer. *Ann Oncol* 2015; 26: 1859–1865.
37. Scher HI, Lu D, Schreiber NA, *et al.* Association of AR-V7 on circulating tumor cells as a treatment-specific biomarker with outcomes and survival in castration-resistant prostate cancer. *JAMA Oncol* 2016; 2: 1441–1449.
38. Maughan BL and Antonarakis ES. Clinical relevance of androgen receptor splice variants in castration-resistant prostate cancer. *Curr Treat Options Oncol* 2015; 16: 57.
39. Kobayashi T, Inoue T, Kamba T, *et al.* Experimental evidence of persistent androgen-receptor-dependency in castration-resistant prostate cancer. *Int J Mol Sci* 2013; 14: 15615–15635.
40. Terada N, Shimizu Y, Kamba T, *et al.* Identification of EP4 as a potential target for the treatment of castration-resistant prostate cancer using a novel xenograft model. *Cancer Res* 2010; 70: 1606–1615.
41. Nishimura K, Nonomura N, Yasunaga Y, *et al.* Low doses of oral dexamethasone for hormone-refractory prostate carcinoma. *Cancer*. 2000; 89: 2570–2576.
42. Inoue T, Ogura K, Kawakita M, *et al.* Effective and safe administration of low-dose estramustine phosphate for castration-resistant prostate cancer. *Clin Genitourin Cancer* 2016; 14: e9–e17.

43. Silberstein JL, Taylor MN and Antonarakis ES. Novel insights into molecular indicators of response and resistance to modern androgen-axis therapies in prostate cancer. *Curr Urol Rep* 2016; 17: 29.
44. Nishimura K, Nonomura N, Satoh E, *et al.* Potential mechanism for the effects of dexamethasone on growth of androgen-independent prostate cancer. *J Natl Cancer Inst* 2001; 93: 1739–1746.
45. Arora VK, Schenkein E, Murali R, *et al.* Glucocorticoid receptor confers resistance to antiandrogens by bypassing androgen receptor blockade. *Cell* 2013; 155: 1309–1322.
46. Narayanan S, Srinivas S and Feldman D. Androgen-glucocorticoid interactions in the era of novel prostate cancer therapy. *Nat Rev Urol* 2016; 13: 47–60.
47. Akamatsu S, Wyatt AW, Lin D, *et al.* The placental gene PEG10 promotes progression of neuroendocrine prostate cancer. *Cell Rep* 2015; 12: 922–936.
48. Mateo J, Carreira S, Sandhu S, *et al.* DNA-repair defects and Olaparib in metastatic prostate cancer. *N Engl J Med* 2015; 373: 1697–1708.
49. Pin E, Fredolini C and Petricoin EF, III. The role of proteomics in prostate cancer research: biomarker discovery and validation. *Clin Biochem* 2013; 46: 524–538.
50. Terada N, Kulkarni P and Getzenberg RH. Cyr61 is a potential prognostic marker for prostate cancer. *Asian J Androl* 2012; 14: 405–408.
51. Terada N, Shiraishi T, Zeng Y, *et al.* Cyr61 is regulated by cAMP-dependent protein kinase with serum levels correlating with prostate cancer aggressiveness. *Prostate* 2012; 72: 966–976.
52. Tanase CP, Codrici E, Popescu ID, *et al.* Prostate cancer proteomics: current trends and future perspectives for biomarker discovery. *Oncotarget* 2017; 8(11): 18497–18512.
53. Goto T, Terada N, Inoue T, *et al.* The expression profile of phosphatidylinositol in high spatial resolution imaging mass spectrometry as a potential biomarker for prostate cancer. *PLoS One*. 2014; 9: e90242.
54. Goto T, Terada N, Inoue T, *et al.* Decreased expression of lysophosphatidylcholine (16:0/OH) in high resolution imaging mass spectrometry independently predicts biochemical recurrence after surgical treatment for prostate cancer. *Prostate* 2015; 75: 1821–1830.
55. Nakayama K, Inoue T, Sekiya S, *et al.* The C-terminal fragment of prostate-specific antigen, a 2331 Da peptide, as a new urinary pathognomonic biomarker candidate for diagnosing prostate cancer. *PLoS One* 2014; 9: e107234.
56. Hegemann M, Stenzl A, Bedke J, *et al.* Liquid biopsy: ready to guide therapy in advanced prostate cancer? *BjU Int* 2016; 118: 855–863.
57. Antonarakis ES, Lu C, Luber B, *et al.* Clinical significance of AR-V7 mRNA detection in circulating tumor cells of men with metastatic castration-resistant prostate cancer treated with first- and second-line abiraterone and enzalutamide. *J Clin Oncol* 2017; 35: 2149–2156.
58. Onstenk W, Sieuwerts AM, Kraan J, *et al.* Efficacy of cabazitaxel in castration-resistant prostate cancer is independent of the presence of AR-V7 in circulating tumor cells. *Eur Urol* 2015; 68: 939–945.
59. Schwarzenbach H, Hoon DS and Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. *Nat Rev Cancer* 2011; 11: 426–437.
60. Schweizer MT and Antonarakis ES. Liquid biopsy: clues on prostate cancer drug resistance. *Sci Transl Med* 2015; 7: 312fs45.
61. Liang DH, Ensor JE, Liu ZB, *et al.* Cell-free DNA as a molecular tool for monitoring disease progression and response to therapy in breast cancer patients. *Breast Cancer Res Treat* 2016; 155: 139–149.
62. Todenhofer T, Azad A, Stewart C, *et al.* AR-V7 transcripts in whole blood RNA of patients with metastatic castration resistant prostate cancer correlate with response to abiraterone acetate. *J Urol* 2017; 197: 135–142.
63. Liu X, Ledet E, Li D, *et al.* A whole blood assay for AR-V7 and ARv567es in patients with prostate cancer. *J Urol* 2016; 196: 1758–1763.
64. Antonarakis ES and Luo J. Blood based detection of androgen receptor splice variants in patients with advanced prostate cancer. *J Urol* 2016; 196: 1606–1607.
65. Tomlins SA, Rhodes DR, Perner S, *et al.* Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science* 2005; 310: 644–648.
66. Hagglof C, Hammarsten P, Stromvall K, *et al.* TMPRSS2-ERG expression predicts prostate cancer survival and associates with stromal biomarkers. *PLoS One*. 2014; 9: e86824.
67. Boormans JL, Hermans KG, Made AC, *et al.* Expression of the androgen-regulated fusion gene TMPRSS2-ERG does not predict response to endocrine treatment in hormone-naive,

- node-positive prostate cancer. *Eur Urol* 2010; 57: 830–835.
68. Leinonen KA, Tolonen TT, Bracken H, *et al.* Association of SPINK1 expression and TMPRSS2: ERG fusion with prognosis in endocrine-treated prostate cancer. *Clin Cancer Res* 2010; 16: 2845–2851.
 69. Cairns P, Okami K, Halachmi S, *et al.* Frequent inactivation of PTEN/MMAC1 in primary prostate cancer. *Cancer Res* 1997; 57: 4997–5000.
 70. Saal LH, Johansson P, Holm K, *et al.* Poor prognosis in carcinoma is associated with a gene expression signature of aberrant PTEN tumor suppressor pathway activity. *Proc Natl Acad Sci USA* 2007; 104: 7564–7569.
 71. Leinonen KA, Saramaki OR, Furusato B, *et al.* Loss of PTEN is associated with aggressive behavior in ERG-positive prostate cancer. *Cancer Epidemiol Biomarkers Prev* 2013; 22: 2333–2344.
 72. Yoshimoto M, Joshua AM, Cunha IW, *et al.* Absence of TMPRSS2:ERG fusions and PTEN losses in prostate cancer is associated with a favorable outcome. *Mod Pathol* 2008; 21: 1451–1460.
 73. Reid AH, Attard G, Ambroisine L, *et al.* Molecular characterisation of ERG, ETV1 and PTEN gene loci identifies patients at low and high risk of death from prostate cancer. *Br J Cancer* 2010; 102: 678–684.
 74. Ahearn TU, Pettersson A, Ebot EM, *et al.* A prospective investigation of PTEN loss and ERG expression in lethal prostate cancer. *J Natl Cancer Inst* 2016; 108(2): pii: djv346.
 75. Attard G, Swennenhuis JF, Olmos D, *et al.* Characterization of ERG, AR and PTEN gene status in circulating tumor cells from patients with castration-resistant prostate cancer. *Cancer Res* 2009; 69: 2912–2918.
 76. Danila DC, Anand A, Sung CC, *et al.* TMPRSS2-ERG status in circulating tumor cells as a predictive biomarker of sensitivity in castration-resistant prostate cancer patients treated with abiraterone acetate. *Eur Urol* 2011; 60: 897–904.
 77. Wyatt AW, Azad AA, Volik SV, *et al.* Genomic alterations in cell-free DNA and enzalutamide resistance in castration-resistant prostate cancer. *JAMA Oncol* 2016; 2: 1598–1606.
 78. Mateo J, Boyses G, Barbieri CE, *et al.* DNA repair in prostate cancer: biology and clinical implications. *Eur Urol* 2017; 71: 417–425.
 79. Robinson D, Van Allen EM, Wu YM, *et al.* Integrative clinical genomics of advanced prostate cancer. *Cell* 2015; 161: 1215–1228.
 80. Pritchard CC, Mateo J, Walsh MF, *et al.* Inherited DNA-repair gene mutations in men with metastatic prostate cancer. *N Engl J Med* 2016; 375: 443–453.
 81. Annala M, Struss WJ, Warner EW, *et al.* Treatment outcomes and tumor loss of heterozygosity in germline DNA repair-deficient prostate cancer. *Eur Urol* 2017; 72: 34–42.
 82. Antonarakis ES. Germline DNA repair mutations and response to hormonal therapy in advanced prostate cancer. *Eur Urol* 2017; 72: 43–44.
 83. Cheng HH, Pritchard CC, Boyd T, *et al.* Biallelic inactivation of BRCA2 in platinum-sensitive metastatic castration-resistant prostate cancer. *Eur Urol* 2016; 69: 992–995.
 84. Schweizer MT, Cheng HH, Tretiakova MS, *et al.* Mismatch repair deficiency may be common in ductal adenocarcinoma of the prostate. *Oncotarget* 2016; 7: 82504–82510.
 85. Steinberger AE, Cotogno P, Ledet EM, *et al.* Exceptional duration of radium-223 in prostate cancer with a BRCA2 mutation. *Clin Genitourin Cancer* 2017; 15: e69–e71.
 86. Teply BA, Kachhap S, Eisenberger MA, *et al.* Extreme response to high-dose testosterone in BRCA2- and ATM-mutated prostate cancer. *Eur Urol* 2017; 71: 499.
 87. Lorente D, Mateo J, Perez-Lopez R, *et al.* Sequencing of agents in castration-resistant prostate cancer. *Lancet Oncol* 2015; 16: e279–e292.
 88. Handy CE and Antonarakis ES. Sequencing treatment for castration-resistant prostate cancer. *Curr Treat Options Oncol* 2016; 17: 64.