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ABOUT COVER

Editorial board member of *World Journal of Clinical Oncology*. After receiving his MD degree from Guangdong Medical University, Dr. Luo attended Department of Pathology, Guangdong Medical University in 2006, and became an Assistant and Associate Professor of Pathology in 2008 and 2013, respectively. From 2017 to now, Dr. Luo began to work in The Second Affiliated Hospital of Southern University of Science and Technology (The Third People's Hospital of Shenzhen). His team investigates molecular mechanisms mediating invasion and stem cell of tumors including nasopharyngeal carcinoma (NPC). Dr. Luo served as the Director of Department of Pathology, The Second Affiliated Hospital of Southern University of Science and Technology.

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REVIEW

Circulating cell-free nucleic acids as prognostic and therapy predictive tools for metastatic castrate-resistant prostate cancer

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Abstract

Metastatic castrate-resistant prostate cancer remains a disease hard to cure, and for this reason predictive tools to monitor disease progression and therapy response are an urgent need. In this respect, liquid biopsy on circulating cell-free nucleic acids represents an interesting strategy based on robust data. The low invasiveness and the possibility to target circulating cell-free tumor deoxyribonucleic acid underline the high specificity, sensitivity and clinical usability of the technique. Moreover, it has been observed that the cell-free tumor deoxyribonucleic acid of metastatic castrate-resistant prostate cancer patients can be representative of the tumor heterogeneity. Cell-free tumor deoxyribonucleic acids express the same behaviors as mutations: Variation in gene copy number or the methylation rate of the tumor tissue. Recently, circulating cell-free ribonucleic acid molecules have emerged as interesting markers to stratify the disease. Due to high-throughput technologies, liquid biopsy on circulating cell-free nucleic acids will soon be utilized in the clinical management of metastatic castrate-resistant prostate cancer patients.

Key words: Metastatic castrate-resistant prostate cancer; Circulating free deoxyribonucleic acid; Cell-free tumor deoxyribonucleic acid; Circulating free ribonucleic acid; Liquid biopsy; Prostate cancer

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Core tip: Among men in industrialized countries, prostate cancer is the most frequent occurring type of cancer and the leading cause of cancer-related deaths. To assure an optimal management of metastatic castrate-resistant prostate cancer patients, specific markers to monitor response to therapies and to predict the clinical outcomes are an urgent need. Liquid biopsy on circulating cell-free deoxyribonucleic acid is able to give useful information about the genetic status of the tumor and the prognosis. Liquid biopsy on circulating cell-free nucleic acids has the potential to integrate clinical data for a personalized management of patients.

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INTRODUCTION

Prostate cancer is the most frequently occurring type of cancer among men in over one-half (105 of 185) of world's countries, and it is the leading cause of cancer-related deaths among men in 46 countries[1]. Although inhibition of androgen receptor (AR) signaling with antiandrogens and conventional chemotherapeutic molecules in metastatic castrate-resistant prostate cancer (mCRPC) patients has increased the 5-year survival rate to 29%, it still remains difficult to cure[2]. In mCRPC, cancer cells adapt to live with very low levels of androgens. Such cells try to use different strategies to independently promote the AR pathway.

After developing the first antiandrogens (flutamide, nilumatide and bicalutamide), there was an urgent need for impeding any agonist activity against wild-type AR. There was a necessity to inhibit wild-type AR recruitment of co-activators and to block the consequent AR binding to deoxyribonucleic acid (DNA), which is a transcription factor for androgen-dependent genes that leads to tumor proliferation[3]. This brought the development of second-generation antiandrogens, which showed a significant improvement in clinical treatment. In fact, in 2012 the Food and Drug Administration approved enzalutamide and abiraterone acetate as second-generation antiandrogens for the treatment of mCRPC, while other second-generation antiandrogens are at different stages of pre-clinical and clinical development[4]. Although these two drugs act on different levels of the AR pathway, the cross-resistance is a common event; resistance to abiraterone and enzalutamide was found to be associated with splicing variants of AR without the ligand-binding domain. Such splice variants encode for a receptor incapable of binding to ligands. It is constitutively active as a transcription factor and capable of promoting the activation of target genes. Because enzalutamide exerts its antitumor activity by binding to the ligand-binding domain of the receptor, the splice variants cannot be targeted by the drug, which generates drug-resistance. Moreover, as the receptor is constitutively activated, it will not be influenced by abiraterone, which inhibits the synthesis of androgens. In this scenario, androgens are not required to activate the pathway, as it is already active[5]. Interestingly, 20%-40% of mCRPC patients not previously treated with chemotherapy are intrinsically resistant to abiraterone acetate or enzalutamide[6].

Thus mCRPC cells evolving into an AR-independent type escape targeted therapies against androgens. Sometimes they can acquire characteristics of neuroendocrine prostate cancer, which is a transformation that occurs in the clinical context of a moderate increment of blood prostate specific antigen (PSA) levels[7]. It is clear that mCRPC progression can have different therapeutic options, but the monitoring of mCRPC is an urgent task for patients' surveillance to anticipate relapse of the disease or to evaluate the efficacy of the therapies. In this respect, we will discuss the emerging role of liquid biopsy for metastatic castrate-resistant prostate cancer patients with a focus on nucleic acids to predict disease progression or therapy efficacy.

LIQUID BIOPSY IN METASTATIC CASTRATE-RESISTANT PROSTATE CANCER

In contrast to traditional tissue biopsy, liquid biopsy involves the analysis of different biological fluids. As technologies advance, the amplification and detection methods have become more and more efficient and sensitive in the target identification, using minute quantities of nucleic acids. As a proof of principle, liquid biopsy can detect various tumor-specific macromolecules to profile and stratify the pathology. Longitudinal monitoring by liquid biopsy could give critical support to clinical management of patients because specific molecular changes are correlated with tumor progression and response to therapies.

Di Nunno *et al*[8] summarized in six points the advantages of liquid biopsy as an innovative approach to prostate cancer: (1) Low invasiveness; (2) Early detection of most aggressive tumors; (3) Early diagnosis of residual tumors or micrometastasis after surgery; (4) Monitoring patient response and/or progression of the disease after therapy, particularly for mCRPC; (5) Prognostication of the response to therapy; and (6) possibility to delineate an accurate genetic profile of the disease directing the analysis towards key mutations correlated with tumor resistance[8].

Many molecular alterations (mutations, amplifications, deletions, alterations in the expression of specific genes or in non-coding RNAs transcription levels) have been correlated with resistance or sensibility against specific treatments. Targets in liquid biopsy can be circulating cell-free nucleic acids. Circulating cell-free tumor DNA (ctDNA) analysis includes detection of circulating free DNA (cfDNA) levels, circulating cell-free DNA integrity (cfDI), methylation rate, mutations and/or aberrations in the copy number of specific genes. For example, amplification of AR and/or the detection of certain driver mutations, *e.g.* deletion of PTEN, can be useful to anticipate an unfavorable clinical outcome[9]. Of interest are the AR mutations, particularly of certain splicing variants (AR-V7), correlating with resistance against second-generation hormone treatments, such as abiraterone acetate and enzalutamide. Moreover, inherited gene mutations that have been found significantly correlated with the prognosis of the disease are eligible for liquid biopsy analysis[10].

Other valuable emerging tumor biomarkers in liquid biopsy are represented by different classes of RNAs, including microRNAs (miRNAs), long non-coding RNA and messenger RNA (mRNA).

ctDNA analysis in mCRPC patients

The total cfDNA concentration was increased in cancer patients and may be a useful tool[11]. However, more information for patient management is derived from the analysis of ctDNA. Table 1 summarizes the main studies investigating cfDNA in mCRPC patients by liquid biopsy. A strategy is to calculate the circulating cfDI, which is the evaluation of the cfDNA fragmentations of repetitive elements such as ALU and LINE-1. It has been observed that in tumors the cfDI value can increase or decrease. The concentration and integrity of circulating cfDNA have been proposed as a clinical tool for the diagnosis of prostate cancer. Khani *et al*[45] proved in Iranian patients that the values of cfDI were significantly higher in patients with prostate cancer *vs* those with benign hyperplasia and healthy individuals. They measured a region of ALU repetitive elements with shorter fragments of 115 bp nested in longer 247 bp ones by quantitative polymerase chain reaction (qPCR); the ratio of 247 bp over the 115 bp gave the integrity DNA index. Additionally, both the DNA concentration and cfDI were found to be elevated in the metastatic conditions[45]. These results were confirmed by Arko-Boham *et al*[46]. Interestingly, cfDI of ALU247/115 had already been found to be higher in metastatic prostate cancer patients *versus* non metastatic ones[40].

In the amount of purified cfDNA, ctDNA can be analyzed by searching gene aberrations such as copy number variations or mutations in target genes. The analysis of genetic alterations of AR, identified through ctDNA in mCRPC patients, that circulating molecules have a significant potential to guide the use of therapies against this receptor. Furthermore, the monitoring of ctDNA levels could result in a powerful way to trace response of tumors and detect the uprising of resistant subclones[6]. In addition to alterations of the AR pathway, other modifications in *TP53, WNT, PI3K* and cell cycle pathway (*RB1* and *CCND1*) genes are important for prognosis and drug-development[7]. It should be noted that the deletion of *PTEN* is found in 40% of patients with mCRPC and loss-of-function mutations in genes of the DNA repair pathway (homologous recombination and mismatch) are expressed in approximately 20% of metastatic prostate cancer[7]. *TP53* gene is very frequently mutated in mCRPC. In particular, *TP53* gain-of-function mutations have been associated with cancer cell

Table 1 Diagnostic and prognostic methods and outcomes of studies investigating cell-free tumor deoxyribonucleic acid in metastatic castrate-resistant prostate cancer by liquid biopsy

Ref.	Methods and patients	Prognostic or predictive outcomes
Ritch *et al*[12], 2019	Plasma, whole exome sequencing. Intron and exon sequencing and copy numbers of selected genes. Mismatched repair deficiency, MMRd mPCa (mostly mCRPC) patients (n = 433)	cfDNA analysis of hypermutations and MMR gene alterations in MSH2, MSH6, MLH1 marked ctDNA and can identify aggressive disease. Mutations in ctDNA were found in PTEN, RB1, TP53 (and, interestingly, no copy number loss) and in AKT1, PI3KCA, CTNNB1, AR-LBD. Compared with a control cohort, ctDNA hypermutation and MMRd correlated with a poor response to AR inhibition and to a shorter survival
Chapman *et al*[13], 2019	Plasma, whole-genome sequencing. Somatic gain of function mutations of *TP53*. mCRPC patients (n = 143)	In cfDNA somatic GOF mutations of *TP53* at codons R175, R248, R273, R282 and G245 were positively and significantly associated with abiraterone and/or enzalutamide progression
Gupta *et al*[14], 2019	Plasma, low pass whole-genome sequencing ULP-WGS. Somatic copy number alteration, mCRPC patients (n = 93)	The SCNA in cfDNA and CTC were mostly concordant (gain in copy number of *FOXA1*, *AR*, and *MYC*, and loss in *BRCA1*, *PTEN*, and *RB1)*. Interestingly, some discordant genomic alterations rarely detected in cfDNA (gain in *MYC* and *BRCA2*) were associated to a worse outcome, *i.e.* MYCN copy number gain correlated with a worse outcome in *AR-V7* negative patients and *BRCA2* copy number gain correlated with a worse outcome in AR-V7 negative patients treated with abiraterone/enzalutamide
Patsch *et al*[15], 2019	Plasma, qPCR of *LINE-1* 297 bp, mCRPC patients before and after docetaxel treatment (n = 15)	In cfDNA, LINE-1 quantity decreased after chemotherapy but without statistical significance
Hahn *et al*[16], 2019	Serum, next generation sequencing, somatic copy number alteration of 69 targeted genes, Metastatic PCa patients (n = 101)	In ctDNA, SCNA significantly correlated with the number of new treatments demonstrating changes of tumor genomic profile after therapies. Interestingly, SCNA did not correlate with time of progression
Qiu *et al*[17], 2019	Plasma, tissue tumor mutational burden by next generation sequencing, targeted gene panel, mCRPC (n = 20)	The ctDNA was identified by two TMB assays: guardant health and the foundation medicine. Between these two assays used to detect ctDNA the results of SNP, Indel, CNV and fusions were concordant. The study evinced a high correlation between cfDNA TMB and the whole exome sequencing of the corresponding tumor tissues. Additionally, there was a good correlation between cfDNA TMB and tissue TMB having high TMB samples; however, the same was not observed for low/medium TMB samples
Torquato *et al*[18], 2019	Plasma, AR alterations, whole genome sequencing, Somatic copy number alteration, mCRPC patients (n = 62)	In ctDNA the SCNA significantly associated with worse survival outcomes of mCRPC patients. At multivariable analysis, missense mutations in AR ligand-binding domain correlated with shorter PFS and *TP53* loss. *PI3KCA* copy number gain or pathogenic missense mutations correlated with shorter OS
De Laere *et al*[19], 2019	Plasma, low pass whole-genome sequencing, and targeted gene-body sequencing. Somatic alterations in *AR* and *TP53*. mCRPC patients (n = 168)	The presence of *AR* and *TP53* alterations in ctDNA was used to determine tumor burden. AR and TP53 alterations were associated with a worse PFS. *TP53* inactivation was an independent prognostic factor outperforming *AR* alterations and *ARV7* expressions was found be useful to stratify patients' risk
Sonpavde *et al*[20], 2019	Plasma, somatic mutations by NGS, mCRPC patients (n = 514)	One or more alterations in ctDNA were found in 94% of patients. Mutations were detected in *TP53* (36%), *AR* (22%), *APC* (10%), *NF1* (9%), *EGFR*, *CTNNB1* and *ARID1A* (6%), *BRCA1*, *BRCA2*, *PIK3CA* (5%). The increase in copy number was found for *AR* (30%), *MYC* (20%), *BRAF* (18%). At multivariate analysis, increase in *MYC* copy number was associated with worse OS
Vandekerkhove *et al*[21], 2019	Plasma, targeted genome, sequencing, somatic copy number alteration, metastatic castrate-sensitive patients (n = 53)	The ctDNA and tissue biopsy showed 80% of concordance for somatic mutations. TP53 was mutated in 50% of patients and truncated mutations in DNA damage repair genes were found in 21%. The cfDNA SCNA was higher in untreated patients than in patients treated with abiraterone acetate, enzalutamide or apalutamide
Mayrhofer *et al*[22], 2018	Plasma, low pass whole-genome sequencing and hybridization-capture targeted sequencing. Somatic copy number alteration, mPCa patients (n = 217)	The SCNA found in ctDNA was used to determine tumor burden and to compare lines of therapies. AR variants, microsatellite instability and *PTEN*, *RB1* and *TP53* inactivation were found in ctDNA demonstrating mirroring of the genetic alterations that mark metastatic cancer
Choudhury *et al*[23], 2018	Plasma, ultra-low pass whole-genome sequencing. Tumor fractions, TFx, measured by computational tool ichorCNA, CRPC patients (n = 140)	TFx was determined in cfDNA. TFx positively correlated with PSA and alkaline phosphatase and significantly correlated with the presence and numbers of bone metastases
Annala *et al*[24], 2018	Plasma, whole-exome sequencing and target capture and	The alterations in *TP53* and *AR* gene truncations measured in ctDNA correlated to resistance to abiraterone and enzalutamide.

	sequencing of selected genes. Variant allele fractions, mCRPC patients (n = 202)	A poor clinical outcome was associated with alterations in ctDNA of *BRCA2* and *ATM* genes
Kohli *et al*[25], 2018	Plasma, dPCR for copy number gain of *AR*, mCRPC patients (n = 92)	In pre chemotherapy patients, the *AR* copy number gain found in ctDNA was associated with a worse outcome
Mehra *et al*[26], 2018	Plasma, cfDNA quantity quant-IT picogreen HS DNA kit and a BioTek microplate spectrophotometer (480ex/520em). mCRPC patients (n = 571)	In multivariable analyses, $\log_{10}$ cfDNA concentration was found to be an independent prognostic variable for rPFS and OS: higher baseline concentrations associated with shorter rPFS and OS following taxane therapy. On the contrary, a decrease in total cfDNA concentration during the first 9 wk of treatment was associated with taxane therapy responsiveness
Seyedolmohadessin *et al*[27], 2018	Plasma, cfDNA quantity NanoDrop, Localized PCa (n = 50), metastatic PCa (n = 26) and healthy controls (n = 10)	The cfDNA level was significantly higher in metastatic PCa patients (19.62 ng/μL) with respect to localized PCa (15.4 ng/μL) and BPH patients (9.5 ng/μL); healthy controls had the lowest value (8.7 ng/μL)
Belic *et al*[28], 2018	Plasma, deep AR sequencing, Illumina MiSeq; whole genome sequencing and targeted sequencing. Somatic copy number alteration, mCRPC patients (n = 65)	In ctDNA, *AR* mutations and copy number alteration were found in most cases. *AR* amplification and *RB1* loss were associated with worse PFS. SCNA was therefore a biomarker for disease progression
Hendriks *et al*[29], 2017	Plasma, Methylation-specific qPCR and copy number of GSTP1 and APC genes, CRPC patients (n = 47), controls (n = 30)	In CRPC patients the cfDNA quantity was significantly higher than age-matched controls. At baseline, *GSTP1* was hypermethylated in patients. Both the copy number of methylated GSTP1 and APC were higher in patients than healthy controls. The increase of cfDNA levels, either each one of the methylated gene copies individually or together (GSTP1 + APC) or together with PSA (GSTP1 + APV + PSA), all correlated with decreased OS
Wyatt *et al*[30], 2017	Plasma, targeted sequencing, Somatic copy number alteration, mCRPC (n = 45)	The study proved a correspondence between SCNA in ctDNA and matched tissues. Such SCNA genes included *AR, BRCA2, ATM, PTEN, PIK3CA, PIK3CB, PIK3R1, TP53*, and *RB1*
Rathkopf *et al*[31], 2017	Plasma, dPCR of 11 relevant AR-ligand binding domain mutations. Non-metastatic CRPC (n = 51), AAP-näive mCRPC (n = 25), post-AAP (n = 21)	In ctDNA, the *AR-LBD* mutations were found to be low at baseline (7.5%) and progression (7.3%). The *AR-LBD* mutations did not correlate with the *de novo* resistance to apalutamide
Goodall *et al*[32], 2017	Plasma, Quant-iT, whole exome sequencing and targeted sequencing. Targeted genes, mCRPC patients (n = 49)	At multivariate analysis, the cfDNA concentration was an independent prognostic biomarker: ≥ 50% reduction in cfDNA levels related to longer rPFS and OS. The ctDNA germline and somatic alterations in *BRCA2* and *PALB2* repair genes were found in ctDNA. All mutations found in the tissue were also detectable in ctDNA
Conteduca *et al*[33], 2017	Plasma, dPCR. Somatic copy number gain of *AR*, mCRPC patients (n = 80)	In ctDNA, the *AR* copy number gain was associated with a worse outcome in patients treated with abiraterone and enzalutamide. Independently from the type of antiandrogen treatment, there was a meaningful correlation among *AR* gain and *TLA/MTV* compared to *AR* non-gained cases (P = 0.001 and P = 0.004, respectively). *AR* copy number and *TLA* were associated with a shorter PFS and OS
Annala *et al*[34], 2017	Plasma, somatic mutations of *BRCA2* gene by qPCR, mCRPC germline-mutated patients (n = 11)	In 10 out of 11 germline mutated patients, biallelic gene loss of *BRCA2* was found in ctDNA. This information help to guide clinicians to the best therapeutic choice
Conteduca *et al*[35], 2017	Plasma, dPCR. Copy number gain of AR, CRPC patients (n = 265)	In ctDNA, the *AR* copy number gain before starting enzalutamide or abiraterone was associated with a decrease in both PFS and OS
Goldstein *et al*[36], 2017	Plasma, NGS AR sequencing and validation by dPCR, somatic alterations in *AR*, mCRPC patients (n = 11)	In ctDNA, the *AR* t (TC > CTC) F877L hotspot was prone to false positive mutations during NGS. Low-abundance mutations need to be verified by highly sensitive PCR, such as dPCR, but amplification conditions must be carefully optimized
Adalsteinsson *et al*[37], 2017	Plasma WES, metastatic PCA PCa patients (n = 520)	There is a concordance between clonal somatic mutations (88%), copy number alterations (80%), mutational signatures and neoantigens between tumor biopsies and cfDNA form 41 patients with ≥ 10% cfDNA
Wyatt *et al*[38], 2016	Plasma, AR copy number qPCR and *AR* deep targeted sequencing, mCRPC patients (n = 65)	In ctDNA, the *AR* mutation and copy number alterations were found in 48% of baseline patients and in 60% patients at disease progression. The *AR* copy number gain (two or more *AR* mutations) and *RB1* loss were associated with worse PFS
Salvi *et al*[39], 2016	Plasma, qPCR. Copy number gain of *AR*, CRPC patients (n = 59)	In ctDNA, the *AR* copy number gain was found in 36% of patients. *AR* copy number gain significantly associated with alkaline

		phosphatase and lactate dehydrogenase. At multivariate analysis, PSA decreasing ≥ 50% and *AR* copy number gain were significantly associated with worse OS and PFS
Fawzy *et al*[40], 2016	Plasma, qPCR of ALU 247bp and ALU115bp, cell-free DNA Integrity, cfDI, metastatic PCa (n = 28), non-metastatic PCa (n = 22), BPH (n = 25), healthy controls (n = 30)	The cfDI levels, measured as ratio ALU247bp/ALU115bp, were significantly higher in metastatic PCa patients *vs* non-metastatic PCa patients, BPH patients and healthy controls
Azad *et al*[41], 2015	Plasma, *AR* qPCR copy number and deep sequencing of *AR-LBD*, mCRPC (n = 62)	In cfDNA, the *AR* copy number gain was associated with enzalutamide resistance; also abiraterone resistance was associated to AR mutations but to a lower extent
Deligezer *et al*[42], 2010	Plasma, qPCR for *Sat-2* gene, PCa-localized (n = 22), locally advanced (n = 11), mCRPC (n = 28)	The average quantity of cfDNA measured by amplification of *Sat2* gene was not significantly different between patients with localized, locally advanced and metastatic disease
Schwarzenbach *et al*[43], 2009	Plasma, somatic LOH for *D6S1631*, *D8S286* and *D9S171* genes by qPCR, PCa patients (n = 69), metastatic PCa patients (n = 12)	In ctDNA, the somatic LOH significantly correlated with the diagnosis of subgroups made of localized and metastasized prostate cancers. ctDNA LOH significantly associated also with the tumor stage
Bastian *et al*[44], 2007	Serum, qPCR for *GSTP1*, *MDR1* and *EBNRB* genes, PCa patients (n = 192)	The levels of cfDNA was found to increase from PCa without recurrence to PCa with recurrence and then to metastatic PCa for all GSTP1, *MDR1 or EBNRB* genes

MMRd: Mismatch repair deficiency; AAP: Acetate and prednisone treatment; AR: Androgen receptor; ARV7: Androgen receptor variant 7; BPH: Benign prostatic hyperplasia; cfDI: Cell-free DNA integrity; cfDNA: Cell-free circulating DNA; CRPC: Castrate-resistant prostate cancer; CTC: Circulating tumor cells; ctDNA: Circulating tumor DNA; dPCR: Digital PCR; GOF: Gain of function; NGS: Next generation sequencing; PFS: Progression-free survival; LP-WGS: Low pass whole-genome sequencing; LOH: Loss of heterogeneity; OS: Overall survival; PCa: Prostate cancer; mCRPC: Metastatic castrate-resistant prostate cancer; mPCa: Metastatic prostate cancer; PSA: Prostate specific antigen; qPCR: Quantitative PCR; rPFS: Radiographic progression-free survival; SCNA: Somatic copy number alteration; scfDNA: Seminal plasma cfDNA; TGS: Deep targeted sequencing; TFx: Tumor fractions; TMB: Tumor mutation burden; ULP-WGS: Ultra low pass whole-genome sequencing; WES: Whole exome sequencing: LBD: Ligand binding domain.

survival and chemoresistance. Interestingly, *TP53* mutations conferring gain of function were related to disease progression and drug resistance after abiraterone or enzalutamide treatments[13].

Different studies offer a comparison between ctDNA and the corresponding metastatic tissue. For example, Wyatt *et al*[30] sequenced 72 clinically relevant genes in 45 cfDNA samples and corresponding tissues obtained during biopsy. The comparison of data concerning alterations in ctDNA with those of the tissues has been demonstrated that for the majority of patients an assay of ctDNA could be enough to identify all the alterations in a metastatic tissue. The authors suggested that with appropriate validation methods, it could be possible to develop DNA-based biomarkers useful in identifying ctDNA for the management of patients with mCRPC. An important advantage of ctDNA is the ability to integrate somatic information from metastatic sites to discover mutation heterogeneity of the tumor, which should be taken into consideration to monitor the tumor stage and its progression. This information could add knowledge to the pathological analysis of the prostate tissue biopsy, which by itself may be not completely representative of the heterogeneous behavior of tumors[30].

ctRNA in prostate cancer patients

The necessity of a multi-parameter approach has been highlighted in different studies. Indeed, important additional knowledge could arise from a simultaneous

investigation of molecules using liquid biopsy (*i.e.* cfDNA) and DNA extracted from circulating tumor cells and cfRNA, with the purpose of building a complete molecular profile[47] to integrate with the clinical data.

RNA evaluation in liquid biopsy represents the next frontier in integrated molecular medicine. Table 2 summarizes the main clinical investigations on ctRNA. Most studies involved prostate cancer patients at diagnosis. Pioneer exploration of RNA molecules in liquid biopsy has involved the analysis of prostate specific membrane antigen mRNA, but only a limited number of newly diagnosed patients were positive[62], whereas the bone morphogenetic protein-6 mRNA, whose upregulation is strongly associated with bone metastasis, was found to be a biomarker for the metastatic disease[42].

Circulating mRNAs are generally degraded by RNases. However, circulating mRNAs are capable of forming complexes with transporters, which are proteins and/or lipids. When they form such complexes, they turn into a relatively stable structure in the blood circulation, thus becoming potentially useful biomarkers[63]. Currently, androgen receptor variants are generally responsible for AR activity, survival and progression of prostate cancer[64]. The *AR-V7* is the only one of the androgen receptor variants observed as a protein, and it is therefore properly defined[3]. Different studies in the literature have shown that *AR-V7* is the main AR variant[65]. The *AR-V7* product is a truncated AR protein, which lacks the C-terminal ligand-binding domain but retains the transactivating N-terminal domain. Consequently, this protein is not capable of binding to ligands but remains a constitutively active transcription factor promoting the activation of target genes promoting cancer progression[65-67]. Antonarakis *et al*[60] evinced that *AR-V7* mRNA is expressed at high levels in circulating tumor cells[60] and is associated with abiraterone and enzalutamide resistance in mCRPC patients[68,69]. Indeed, Joncas *et al*[48] recently found blood levels of *AR-V7* mRNA, which was shown to be correlated with response and resistance to abiraterone in mCRPC patients, demonstrating its potential as predictive biomarker[48]. Particularly worthy of note, upregulation of the programmed death-ligand *PD-L1* mRNA causes cancer cells to able to evade the host immune system[50].

In most patients, altered levels of miRNAs have been found, such as miR-21, miR-221, miR-1290 and miR-375. Such differential expression compared to healthy controls have been associated with different prognostic outcomes in mCRPC patients. For example, a potential diagnostic and prognostic role played by miR-141 has been suggested. miR-141 levels have been found to be progressively increased from hypertrophy of the prostate to prostate cancer and to the metastatic disease[70]. Interestingly, the quantification of miR-141 in the liquid biopsy by droplet digital PCR has been described[71]. However, a recent study showed that miR-18a has the highest potency to discriminate between healthy individuals and cancer patients, whereas miR-221 discriminated between patients with localized disease from those with metastasis. miR-141 did not show the same potency[72].

FUTURE PERSPECTIVES

Liquid biopsy represents an attractive field of research for many types of cancer, including prostate adenocarcinoma. In particular, liquid biopsy has been proven to provide support in therapeutic planning for patients with mCRPC, allowing detection of molecular changes in cell-free nucleic acids (*i.e.* DNA and RNA) that are associated with tumor progression and response or resistance to different drugs. The low invasiveness is particularly relevant because of the possibility of repeating the analyses frequently over time, allowing longitudinal monitoring of patients. In the near future, liquid biopsy will lead to a deeper understanding of the metastatic evolution of prostate cancer with the possibility of developing new targeted therapies in the perspective of an even more personalized oncology.

The analysis of ctDNA appears to be the most promising tool to monitor cancer diseases. In fact, ctDNA is the only target recommended by the Food and Drug Administration and the European Medicines Agency for cancer diagnosis and to monitor the efficacy of treatments. For prostate cancer, ctDNA is a very interesting biomarker for the anticipation of progression-free survival and overall survival in response to therapies and for improving the clinical management of patients avoiding overtreatments. The high concordance between ctDNA genomic alterations and those found in tumor tissue biopsies strongly supports the potential of liquid biopsy to integrate clinical data and improve patient management. The next generation

Table 2 Diagnostic and prognostic outcomes methods of studies investigating circulating tumor ribonucleic acid in prostate cancer by liquid biopsy

Ref.	Methods and patients	Prognostic or predictive outcomes
Joncas *et al*[48], 2019	Plasma dPCR, *AR-7* mRNA, PCa patients (n = 35)	*AR-V7* mRNA expression was associated with shorter time to progression (median, 16.0 *vs* 28.0 mo; P = 0.0499)
Mohammadi Torbati *et al*[49], 2019	Serum qPCR, miR-20A, miR-26A, PCa patients (n = 40), healthy controls (n = 40)	In PCa samples miR-20A was significantly upregulated compared to healthy controls. On the other hand, there was no significant difference in the levels of pre- and post-operation miR-26A compared to controls
Ishiba *et al*[50], 2018	Plasma dPCR, *PDL-1* mRNA, PCa patients (n = 88)	*PD-L1* mRNA was detected and quantified in ctRNA of cancer patients. Interestingly, there was a comparison between expression of PD-L1 protein in tumor tissues and PD-L1 gene expression in plasma of cancer patients
Wang *et al*[51], 2018	Plasma qPCR, SAP30L-AS1 and SChLAP1 lncRNAs, PCa patients (n = 34), BPH patients (n = 46), Healthy controls (n = 30)	SAP30L-AS1 lncRNAs levels were upregulated in BPH and SChLAP1 lncRNAs levels were significantly higher in PCa than in BPH and healthy controls. The area under the ROC curve indicated that SAP30L-AS1 and SChLAP1 lncRNA had an adequate diagnostic value different from PCa and controls
Zedan *et al*[52], 2018	Plasma qPCR, miR-93, miR-221, miR-125b, miR-93, PCa patients (n = 149)	Significantly lower levels of miRNA-93 and miRNA-221 in the follow-up of patients *vs* baseline $z = -2.738$, P = 0.006, and $z = -4.498$, $P < 0.001$, respectively. Similarly, miRNA-125b was significantly lower in the observational cohort ($z = -2.656$, P = 0.008). There was a correlation between both miRNA-125b and miRNA-221 with risk assessment r = 0.23, P = 0.015 and r = 0.203, P = 0.016, respectively. However, miRNA-93 was significantly correlated with prostatectomy Gleason score (r = 0.276, P = 0.0576)
Farran *et al*[53], 2018	Plasma qPCR, miRNA signature, PCa patients (n = 114)	Aggressiveness of PCa could be segregated based on circulating miRNA signature consisting of an interaction between a combination of two miRNAs (miR-17/miR-192) and an independent miRNA (miR-181a)
Liu *et al*[54], 2018	Plasma qPCR, miR-223, miR-24, miR-375, PCa patients (n = 329)	Patients could be significantly reclassified using a 3-miR (miRNA-223, miRNA-24 and miRNA-375) score (training OR 2.72, 95%CI 1.50e 4.94 and validation OR 3.70, 95%CI 1.29e 10.6)
Adalsteinsson *et al*[37], 2017	Plasma WES, Metastatic PCa patients (n = 520)	There is a concordance between clonal somatic mutations (88%), copy number alterations (80%), mutational signatures and neoantigens between tumor biopsies and cfDNA form 41 patients with ≥ 10% cfDNA
Albitar *et al*[55], 2017	Urine and plasma qPCR, mRNAs panel, PCa patients (n = 306)	The urine/plasma biomarker test, evaluating the mRNA levels of PCa-specific gene such as *PDLIM5, HSPD1, PSA, IMPDH2, PCA3,TMPRSS2, ERG, UAP1, PTEN, AR*, the housekeeping *B2M* and *GAPDH* genes, accurately predicted high-grade cancer with sensitivity at 92%-97%, while core-biopsy sensitivity was 78%
Endzeliņš *et al*[56], 2017	Plasma qPCR, miR-375, miR-200-3p, miR-21-5p, miRNA Let-7a-5p, PCa patients (n = 50), BPH patients (n = 22)	miR-375 could be used to differentiate between PCa and BPH patients when analyzed in whole plasma, while miR-200-3p and miR-21-5p performed better in EVs. Let-7a-5p could be used to differentiate PCa patients, with Gleason score ≥ 8 *vs* ≤ 6
McDonald *et al*[57], 2017	Plasma qPCR, miRNA panel, PCa patients (n = 134)	miR-381, miR-34a, miR-523, miR-365, miR-122, miR-375, miR-1255b, miR-34b, miR-450b-5p, and miR-639 were the most statistically significant miRNA after adjusting for age (P values ≤ 0.05)
Alhasan *et al*[58], 2016	Plasma Scano-miR, miRNA panel, very high risk, PCa patients (n = 9), Low risk, PCa patients (n = 9), and healthy controls (n = 10)	miR-200c, miR-605, miR-135a, miR-433, and miR-106a were identified as useful for differentiating indolent and aggressive forms of PCa
Yan *et al*[59], 2015	Urinary qPCR, TSPAN13 and S100A9 mRNAs, PCa patients (n = 129), BPH patients (n = 105)	qPCR was used to measure urinary nucleic acid levels and tissue mRNA expression. The TSPAN13 and S100A9 mRNA ratio was selected to determine the diagnostic value of urinary nucleic acids in PCa (P = 0.037). It was significantly higher in PCA than in BPH in the mRNA and nucleic acid cohort analyses ($P < 0.001$ and P = 0.013, respectively). ROC analysis showed that the area under the ROC curve was 0.898 and 0.676 in tissue mRNA cohort and urinary nucleic acid cohort, respectively. This ratio could have a strong potential as a diagnostic PCa marker
Antonarakis *et al*[60], 2014	Serum qPCR, AR-V7 mRNA, PCa enzalutamide-treated patients (n = 31),	AR-V7 mRNA detectable (positive) patients receiving enzalutamide had lower PSA response rates compared to AR-V7 mRNA not detectable (negative) patients (0% *vs* 53%, P = 0.004) and shorter PSA PFS (median, 1.4 mo *vs* 6.0 mo; $P < 0.001$), clinical or radiological PFS (median, 2.1 mo *vs* 6.1 mo; $P < 0.001$), and OS (median, 5.5 mo *vs* not reached;

	PCa abiraterone-treated patients (n = 31)	P = 0.002). Similarly, AR-V7 mRNA positive patients, receiving abiraterone had lower PSA response rates compared to AR-V7 mRNA negative patients (0% *vs* 68%, P = 0.004) and shorter PSA PFS (median, 1.3 mo *vs* not reached; P < 0.001), clinical or radiological PFS (median, 2.3 mo *vs* not reached; P < 0.001), and OS (median, 10.6 mo *vs* not reached, P = 0.006)
Korzeniewski *et al*[61], 2014	Urine qPCR, miR-483-5p, PCa patients (n = 71), healthy controls (n = 18)	miR-483-5p was expressed at higher levels in PCa than in control
Deligezer *et al*[42], 2010	Plasma qPCR, cBMP6 mRNA, Local PCa patients (n = 22), local advanced PCa patients (n = 11) or mCRPC patients (n = 28)	The levels of cBMP6 mRNA in patients with metastatic disease were higher than those in patients with localized disease (P = 0.001) or in patients with local advanced disease (P = 0.05)
Papadopoulou *et al*[62], 2006	PBMC and plasma qPCR, PSMA mRNA, newly diagnosed PCa patients (n = 12), under therapy PCa patients (n = 4)	Among the newly diagnosed patients 4/12 (33.3%) had positive mRNA for PSMA in plasma, whereas only 2/12 (16.7%) had positive PSMA mRNA in PBMC. Among under therapy PCa patients, three (15.8%) were positive for PSMA mRNA in plasma, while only one (5.3%) was positive in PBMC. Furthermore, > 60% of PCa had elevated levels of cfDNA

AR: Androgen receptor; AR-V7: Androgen-receptor splice variant 7; BPH: Benign prostatic hyperplasia; cfDNA: Cell free DNA; dPCR: Digital polymerase chain reaction; lncRNAs: Exosomal circulating long non-coding RNAs; mCRPC: Metastatic castrate-resistant prostate cancer; PSA: Prostate specific antigen; WES: Whole exome sequencing; PFS: Progression-free survival; OS: Overall survival; PBMC: Peripheral blood mononuclear cells; PCa: Prostate cancer; qPCR: Quantitative polymerase chain reaction; lncRNA: long non-coding RNA; PSMA: Prostate specific membrane antigen; BMP-6: Bone morphogenetic protein-6.

sequencing of cfDNA has demonstrated the potential for the follow-up of the mutational changes of the tumor by being able to identify all its heterogeneity and to anticipate drug resistance. Moreover, many affordable high-throughput technologies, *e.g.* digital PCR, are now available to precisely detect the copy number variations of selected target genes (*e.g. AR, TP53, BRCA2, PIK3CA*) that are relevant for the progression of the disease and in response to therapies.

The potential usefulness of cfRNAs in mCRPC is emerging, especially as additional markers for aggressiveness and metastasis. Many of the studies in cfRNA involved miRNA analysis, but more recently even other classes of non-coding RNA have been explored, such as long non-coding RNA. In the clinic the potential use of cfRNA analysis could implement information about the staging of the disease, but it might be useful to discriminate indolent *vs* aggressive prostate cancer.

Liquid biopsy data offers robust evidence to consider cell-free nucleic acid analysis useful to improve the clinical management of mCRPC patients. In this new approach the use of PSA as a biomarker must be considered. PSA is the biomarker approved for men by the Food and Drug Administration in 1986. From then on, it has been widely used to predict incidence and recurrence of prostate cancer, despite its poor specificity. However, in mCRPC the PSA seems to be more specific as a biomarker than in the onset of prostate cancer; its increase is related to cancer progression[73]. The significance of PSA measurements in mCRPC is still interesting for the scientific community. For example, Aggarwal *et al*[74] recently demonstrated that low PSA secretion levels can stratify mCRPC patients with treatment-emergent small-cell neuroendocrine prostate cancer. In fact, low PSA secretors showed high treatment-emergent small-cell neuroendocrine prostate cancer, *RB1* and *TP53* loss and low AR transcription. In

addition, overall survival and progression-free survival were shorter in the low PSA secretor group[74]. In a retrospective study, Buttigliero *et al*[75] showed that early PSA drop was related to a better overall survival and progression-free survival in mCRPC patients treated with abiraterone or enzalutamide (docetaxel-naïve or post-docetaxel setting). Finally, a mathematical model of PSA dynamics has been proposed to predict individual response to intermittent androgen deprivation therapy[76].

CONCLUSION

In our opinion, PSA can play an important role as a biomarker for the management of mCRPC patients. However, PSA measurements could maintain some limitations due to the high individual variability. Liquid biopsy on circulating cell-free nucleic acid offers the same low invasiveness but important molecular details on each specific tumor heterogeneity evolution. In conclusion, liquid biopsy on circulating cell-free nucleic acid along with PSA measurements and other clinical data can assure the best treatment decision-making for mCRPC patients.

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REFERENCES

1 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]

2 **Kantoff PW**, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, Redfern CH, Ferrari AC, Dreicer R, Sims RB, Xu Y, Frohlich MW, Schellhammer PF; IMPACT Study Investigators. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med* 2010; **363**: 411-422 [PMID: 20818862 DOI: 10.1056/NEJMoa1001294]

3 **Wadosky KM**, Koochekpour S. Androgen receptor splice variants and prostate cancer: From bench to bedside. *Oncotarget* 2017; **8**: 18550-18576 [PMID: 28077788 DOI: 10.18632/oncotarget.14537]

4 **Rice MA**, Malhotra SV, Stoyanova T. Second-Generation Antiandrogens: From Discovery to Standard of Care in Castration Resistant Prostate Cancer. *Front Oncol* 2019; **9**: 801 [PMID: 31555580 DOI: 10.3389/fonc.2019.00801]

5 **Bettegowda C**, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, Bartlett BR, Wang H, Luber B, Alani RM, Antonarakis ES, Azad NS, Bardelli A, Brem H, Cameron JL, Lee CC, Fecher LA, Gallia GL, Gibbs P, Le D, Giuntoli RL, Goggins M, Hogarty MD, Holdhoff M, Hong SM, Jiao Y, Juhl HH, Kim JJ, Siravegna G, Laheru DA, Lauricella C, Lim M, Lipson EJ, Marie SK, Netto GJ, Oliner KS, Olivi A, Olsson L, Riggins GJ, Sartore-Bianchi A, Schmidt K, Shih lM, Oba-Shinjo SM, Siena S, Theodorescu D, Tie J, Harkins TT, Veronese S, Wang TL, Weingart JD, Wolfgang CL, Wood LD, Xing D, Hruban RH, Wu J, Allen PJ, Schmidt CM, Choti MA, Velculescu VE, Kinzler KW, Vogelstein B, Papadopoulos N, Diaz LA Jr. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med* 2014; **6**: 224ra24 [PMID: 24553385 DOI: 10.1126/scitranslmed.3007094]

6 **Ritch E**, Wyatt AW. Predicting therapy response and resistance in metastatic prostate cancer with circulating tumor DNA. *Urol Oncol* 2018; **36**: 380-384 [PMID: 29248429 DOI: 10.1016/j.urolonc.2017.11.017]

7 **Friedlander TW**, Pritchard CC, Beltran H. Personalizing Therapy for Metastatic Prostate Cancer: The Role of Solid and Liquid Tumor Biopsies. *Am Soc Clin Oncol Educ Book* 2017; **37**: 358-369 [PMID: 28561699 DOI: 10.1200/EDBK_175510]

8 **Di Nunno V**, Gatto L, Santoni M, Cimadamore A, Lopez-Beltran A, Cheng L, Scarpelli M, Montironi R, Massari F. Recent Advances in Liquid Biopsy in Patients With Castration Resistant Prostate Cancer. *Front Oncol* 2018; **8**: 397 [PMID: 30319966 DOI: 10.3389/fonc.2018.00397]

9 **Lu YT**, Delijani K, Mecum A, Goldkorn A. Current status of liquid biopsies for the detection and management of prostate cancer. *Cancer Manag Res* 2019; **11**: 5271-5291 [PMID: 31239778 DOI: 10.2147/CMAR.S170380]

10 **Pritchard CC**, Mateo J, Walsh MF, De Sarkar N, Abida W, Beltran H, Garofalo A, Gulati R, Carreira S, Eeles R, Elemento O, Rubin MA, Robinson D, Lonigro R, Hussain M, Chinnaiyan A, Vinson J, Filipenko J, Garraway L, Taplin ME, AlDubayan S, Han GC, Beightol M, Morrissey C, Nghiem B, Cheng HH, Montgomery B, Walsh T, Casadei S, Berger M, Zhang L, Zehir A, Vijai J, Scher HI, Sawyers C, Schultz N, Kantoff PW, Solit D, Robson M, Van Allen EM, Offit K, de Bono J, Nelson PS. Inherited DNA-Repair Gene Mutations in Men with Metastatic Prostate Cancer. *N Engl J Med* 2016; **375**: 443-453 [PMID: 27433846 DOI: 10.1056/NEJMoa1603144]

11 **Sobhani N**, Generali D, Zanconati F, Bortul M, Scaggiante B. Cell-free DNA integrity for the monitoring of breast cancer: Future perspectives? *World J Clin Oncol* 2018;

9: 26-32 [PMID: 29651384 DOI: 10.5306/wjco.v9.i2.26]

12 **Ritch E**, Fu SYF, Herberts C, Wang G, Warner EW, Schönlau E, Taavitsainen S, Murtha AJ, Vandekerkhove G, Beja K, Loktionova Y, Khalaf D, Fazli L, Kushnir I, Ferrario C, Hotte S, Annala M, Chi KN, Wyatt AW. Identification of Hypermutation and Defective Mismatch Repair in ctDNA from Metastatic Prostate Cancer. *Clin Cancer Res* 2020; **26**: 1114-1125 [PMID: 31744831 DOI: 10.1158/1078-0432.CCR-19-1623]

13 **Chapman L**, Ledet EM, Barata PC, Cotogno P, Manogue C, Moses M, Christensen BR, Steinwald P, Ranasinghe L, Layton JL, Lewis BE, Sartor O. TP53 Gain-of-Function Mutations in Circulating Tumor DNA in Men With Metastatic Castration-Resistant Prostate Cancer. *Clin Genitourin Cancer* 2020; **18**: 148-154 [PMID: 31822380 DOI: 10.1016/j.clgc.2019.10.022]

14 **Gupta S**, Hovelson DH, Kemeny G, Halabi S, Foo WC, Anand M, Somarelli JA, Tomlins SA, Antonarakis ES, Luo J, Dittamore RV, George DJ, Rothwell C, Nanus DM, Armstrong AJ, Gregory SG. Discordant and heterogeneous clinically relevant genomic alterations in circulating tumor cells vs plasma DNA from men with metastatic castration resistant prostate cancer. *Genes Chromosomes Cancer* 2020; **59**: 225-239 [PMID: 31705765 DOI: 10.1002/gcc.22824]

15 **Patsch K**, Matasci N, Soundararajan A, Diaz P, Agus DB, Ruderman D, Gross ME. Monitoring dynamic cytotoxic chemotherapy response in castration-resistant prostate cancer using plasma cell-free DNA (cfDNA). *BMC Res Notes* 2019; **12**: 275 [PMID: 31092276 DOI: 10.1186/s13104-019-4312-2]

16 **Hahn AW**, Stenehjem D, Nussenzveig R, Carroll E, Bailey E, Batten J, Maughan BL, Agarwal N. Evolution of the genomic landscape of circulating tumor DNA (ctDNA) in metastatic prostate cancer over treatment and time. *Cancer Treat Res Commun* 2019; **19**: 100120 [PMID: 30743187 DOI: 10.1016/j.ctarc.2019.100120]

17 **Qiu P**, Poehlein CH, Marton MJ, Laterza OF, Levitan D. Measuring Tumor Mutational Burden (TMB) in Plasma from mCRPC Patients Using Two Commercial NGS Assays. *Sci Rep* 2019; **9**: 114 [PMID: 30643180 DOI: 10.1038/s41598-018-37128-y]

18 **Torquato S**, Pallavajjala A, Goldstein A, Toro PV, Silberstein JL, Lee J, Nakazawa M, Waters I, Chu D, Shinn D, Groginski T, Hughes RM, Simons BW, Khan H, Feng Z, Carducci MA, Paller CJ, Denmeade SR, Kressel B, Eisenberger MA, Antonarakis ES, Trock BJ, Park BH, Hurley PJ. Genetic Alterations Detected in Cell-Free DNA Are Associated With Enzalutamide and Abiraterone Resistance in Castration-Resistant Prostate Cancer. *JCO Precis Oncol* 2019; **3** [PMID: 31131348 DOI: 10.1200/PO.18.00227]

19 **De Laere B**, Oeyen S, Mayrhofer M, Whitington T, van Dam PJ, Van Oyen P, Ghysel C, Ampe J, Ost P, Demey W, Hoekx L, Schrijvers D, Brouwers B, Lybaert W, Everaert EG, De Maeseneer D, Strijbos M, Bols A, Fransis K, Beije N, de Kruijff IE, van Dam V, Brouwer A, Goossens D, Heyrman L, Van den Eynden GG, Rutten A, Del Favero J, Rantalainen M, Rajan P, Sleijfer S, Ullén A, Yachnin J, Grönberg H, Van Laere SJ, Lindberg J, Dirix LY. *TP53* Outperforms Other Androgen Receptor Biomarkers to Predict Abiraterone or Enzalutamide Outcome in Metastatic Castration-Resistant Prostate Cancer. *Clin Cancer Res* 2019; **25**: 1766-1773 [PMID: 30209161 DOI: 10.1158/1078-0432.CCR-18-1943]

20 **Sonpavde G**, Agarwal N, Pond GR, Nagy RJ, Nussenzveig RH, Hahn AW, Sartor O, Gourdin TS, Nandagopal L, Ledet EM, Naik G, Armstrong AJ, Wang J, Bilen MA, Gupta S, Grivas P, Pal SK, Lanman RB, Talasaz A, Lilly MB. Circulating tumor DNA alterations in patients with metastatic castration-resistant prostate cancer. *Cancer* 2019; **125**: 1459-1469 [PMID: 30620391 DOI: 10.1002/cncr.31959]

21 **Vandekerkhove G**, Struss WJ, Annala M, Kallio HML, Khalaf D, Warner EW, Herberts C, Ritch E, Beja K, Loktionova Y, Hurtado-Coll A, Fazli L, So A, Black PC, Nykter M, Tammela T, Chi KN, Gleave ME, Wyatt AW. Circulating Tumor DNA Abundance and Potential Utility in De Novo Metastatic Prostate Cancer. *Eur Urol* 2019; **75**: 667-675 [PMID: 30638634 DOI: 10.1016/j.eururo.2018.12.042]

22 **Mayrhofer M**, De Laere B, Whitington T, Van Oyen P, Ghysel C, Ampe J, Ost P, Demey W, Hoekx L, Schrijvers D, Brouwers B, Lybaert W, Everaert E, De Maeseneer D, Strijbos M, Bols A, Fransis K, Oeyen S, van Dam PJ, Van den Eynden G, Rutten A, Aly M, Nordström T, Van Laere S, Rantalainen M, Rajan P, Egevad L, Ullén A, Yachnin J, Dirix L, Grönberg H, Lindberg J. Cell-free DNA profiling of metastatic prostate cancer reveals microsatellite instability, structural rearrangements and clonal hematopoiesis. *Genome Med* 2018; **10**: 85 [PMID: 30458854 DOI: 10.1186/s13073-018-0595-5]

23 **Choudhury AD**, Werner L, Francini E, Wei XX, Ha G, Freeman SS, Rhoades J, Reed SC, Gydush G, Rotem D, Lo C, Taplin ME, Harshman LC, Zhang Z, O'Connor EP, Stover DG, Parsons HA, Getz G, Meyerson M, Love JC, Hahn WC, Adalsteinsson VA. Tumor fraction in cell-free DNA as a biomarker in prostate cancer. *JCI Insight* 2018; **3** [PMID: 30385733 DOI: 10.1172/jci.insight.122109]

24 **Annala M**, Vandekerkhove G, Khalaf D, Taavitsainen S, Beja K, Warner EW, Sunderland K, Kollmannsberger C, Eigl BJ, Finch D, Oja CD, Vergidis J, Zulfiqar M, Azad AA, Nykter M, Gleave ME, Wyatt AW, Chi KN. Circulating Tumor DNA Genomics Correlate with Resistance to Abiraterone and Enzalutamide in Prostate Cancer. *Cancer Discov* 2018; **8**: 444-457 [PMID: 29367197 DOI: 10.1158/2159-8290.CD-17-0937]

25 **Kohli M**, Li J, Du M, Hillman DW, Dehm SM, Tan W, Carlson R, Campion MB, Wang L, Wang L, Zhang H, Zhang P, Kilari D, Huang CC, Wang L. Prognostic association of plasma cell-free DNA-based androgen receptor amplification and circulating tumor cells in pre-chemotherapy metastatic castration-resistant prostate cancer patients. *Prostate Cancer Prostatic Dis* 2018; **21**: 411-418 [PMID: 29858592 DOI: 10.1038/s41391-018-0043-z]

26 **Mehra N**, Dolling D, Sumanasuriya S, Christova R, Pope L, Carreira S, Seed G, Yuan W, Goodall J, Hall E, Flohr P, Boysen G, Bianchini D, Sartor O, Eisenberger MA, Fizazi K, Oudard S, Chadjaa M, Macé S, de Bono JS. Plasma Cell-free DNA Concentration and Outcomes from Taxane Therapy in Metastatic Castration-resistant Prostate Cancer from Two Phase III Trials (FIRSTANA and PROSELICA). *Eur Urol* 2018; **74**: 283-291 [PMID: 29500065 DOI: 10.1016/j.eururo.2018.02.013]

27 **Seyedolmohadessin SM**, Akbari MT, Nourmohammadi Z, Basiri A, Pourmand G. Assessing the Diagnostic Value of Plasma-Free DNA in Prostate Cancer Screening. *Iran Biomed J* 2018; **22**: 331-337 [PMID: 29475366 DOI: 10.29252/ibj.22.5.331]

28 **Belic J**, Graf R, Bauernhofer T, Cherkas Y, Ulz P, Waldispuehl-Geigl J, Perakis S, Gormley M, Patel J, Li

W, Geigl JB, Smirnov D, Heitzer E, Gross M, Speicher MR. Genomic alterations in plasma DNA from patients with metastasized prostate cancer receiving abiraterone or enzalutamide. *Int J Cancer* 2018; **143**: 1236-1248 [PMID: 29574703 DOI: 10.1002/ijc.31397]

29 **Hendriks RJ**, Dijkstra S, Smit FP, Vandersmissen J, Van de Voorde H, Mulders PFA, van Oort IM, Van Criekinge W, Schalken JA. Epigenetic markers in circulating cell-free DNA as prognostic markers for survival of castration-resistant prostate cancer patients. *Prostate* 2018; **78**: 336-342 [PMID: 29330943 DOI: 10.1002/pros.23477]

30 **Wyatt AW**, Annala M, Aggarwal R, Beja K, Feng F, Youngren J, Foye A, Lloyd P, Nykter M, Beer TM, Alumkal JJ, Thomas GV, Reiter RE, Rettig MB, Evans CP, Gao AC, Chi KN, Small EJ, Gleave ME. Concordance of Circulating Tumor DNA and Matched Metastatic Tissue Biopsy in Prostate Cancer. *J Natl Cancer Inst* 2017; **709** [PMID: 29206995 DOI: 10.1093/jnci/djx118]

31 **Rathkopf DE**, Smith MR, Ryan CJ, Berry WR, Shore ND, Liu G, Higano CS, Alumkal JJ, Hauke R, Tutrone RF, Saleh M, Chow Maneval E, Thomas S, Ricci DS, Yu MK, de Boer CJ, Trinh A, Kheoh T, Bandekar R, Scher HI, Antonarakis ES. Androgen receptor mutations in patients with castration-resistant prostate cancer treated with apalutamide. *Ann Oncol* 2017; **28**: 2264-2271 [PMID: 28633425 DOI: 10.1093/annonc/mdx283]

32 **Goodall J**, Mateo J, Yuan W, Mossop H, Porta N, Miranda S, Perez-Lopez R, Dolling D, Robinson DR, Sandhu S, Fowler G, Ebbs B, Flohr P, Seed G, Rodrigues DN, Boysen G, Bertan C, Atkin M, Clarke M, Crespo M, Figueiredo I, Riisnaes R, Sumanasuriya S, Rescigno P, Zafeiriou Z, Sharp A, Tunariu N, Bianchini D, Gillman A, Lord CJ, Hall E, Chinnaiyan AM, Carreira S, de Bono JS; TOPARP-A investigators. Circulating Cell-Free DNA to Guide Prostate Cancer Treatment with PARP Inhibition. *Cancer Discov* 2017; **7**: 1006-1017 [PMID: 28450425 DOI: 10.1158/2159-8290.CD-17-0261]

33 **Conteduca V**, Scarpi E, Caroli P, Salvi S, Lolli C, Burgio SL, Menna C, Schepisi G, Testoni S, Gurioli G, Paganelli G, Casadio V, Matteucci F, De Giorgi U. Circulating androgen receptor combined with 18F-fluorocholine PET/CT metabolic activity and outcome to androgen receptor signalling-directed therapies in castration-resistant prostate cancer. *Sci Rep* 2017; **7**: 15541 [PMID: 29138500 DOI: 10.1038/s41598-017-15928-y]

34 **Annala M**, Struss WJ, Warner EW, Beja K, Vandekerkhove G, Wong A, Khalaf D, Seppälä IL, So A, Lo G, Aggarwal R, Small EJ, Nykter M, Gleave ME, Chi KN, Wyatt AW. Treatment Outcomes and Tumor Loss of Heterozygosity in Germline DNA Repair-deficient Prostate Cancer. *Eur Urol* 2017; **72**: 34-42 [PMID: 28259476 DOI: 10.1016/j.eururo.2017.02.023]

35 **Conteduca V**, Wetterskog D, Sharabiani MTA, Grande E, Fernandez-Perez MP, Jayaram A, Salvi S, Castellano D, Romanel A, Lolli C, Casadio V, Gurioli G, Amadori D, Font A, Vazquez-Estevez S, González Del Alba A, Mellado B, Fernandez-Calvo O, Méndez-Vidal MJ, Climent MA, Duran I, Gallardo E, Rodriguez A, Santander C, Sáez MI, Puente J, Gasi Tandefelt D, Wingate A, Dearnaley D; PREMIERE Collaborators; Spanish Oncology Genitourinary Group, Demichelis F, De Giorgi U, Gonzalez-Billalabeitia E, Attard G. Androgen receptor gene status in plasma DNA associates with worse outcome on enzalutamide or abiraterone for castration-resistant prostate cancer: a multi-institution correlative biomarker study. *Ann Oncol* 2017; **28**: 1508-1516 [PMID: 28472366 DOI: 10.1093/annonc/mdx155]

36 **Goldstein A**, Toro PV, Lee J, Silberstein JL, Nakazawa M, Waters I, Cravero K, Chu D, Cochran RL, Kim M, Shinn D, Torquato S, Hughes RM, Pallavajjala A, Carducci MA, Paller CJ, Denmeade SR, Kressel B, Trock BJ, Eisenberger MA, Antonarakis ES, Park BH, Hurley PJ. Detection fidelity of AR mutations in plasma derived cell-free DNA. *Oncotarget* 2017; **8**: 15651-15662 [PMID: 28152506 DOI: 10.18632/oncotarget.14926]

37 **Adalsteinsson VA**, Ha G, Freeman SS, Choudhury AD, Stover DG, Parsons HA, Gydush G, Reed SC, Rotem D, Rhoades J, Loginov D, Livitz D, Rosebrock D, Leshchiner I, Kim J, Stewart C, Rosenberg M, Francis JM, Zhang CZ, Cohen O, Oh C, Ding H, Polak P, Lloyd M, Mahmud S, Helvie K, Merrill MS, Santiago RA, O'Connor EP, Jeong SH, Leeson R, Barry RM, Kramkowski JF, Zhang Z, Polacek L, Lohr JG, Schleicher M, Lipscomb E, Saltzman A, Oliver NM, Marini L, Waks AG, Harshman LC, Tolaney SM, Van Allen EM, Winer EP, Lin NU, Nakabayashi M, Taplin ME, Johannessen CM, Garraway LA, Golub TR, Boehm JS, Wagle N, Getz G, Love JC, Meyerson M. Scalable whole-exome sequencing of cell-free DNA reveals high concordance with metastatic tumors. *Nat Commun* 2017; **8**: 1324 [PMID: 29109393 DOI: 10.1038/s41467-017-00965-y]

38 **Wyatt AW**, Azad AA, Volik SV, Annala M, Beja K, McConeghy B, Haegert A, Warner EW, Mo F, Brahmbhatt S, Shukin R, Le Bihan S, Gleave ME, Nykter M, Collins CC, Chi KN. Genomic Alterations in Cell-Free DNA and Enzalutamide Resistance in Castration-Resistant Prostate Cancer. *JAMA Oncol* 2016; **2**: 1598-1606 [PMID: 27148695 DOI: 10.1001/jamaoncol.2016.0494]

39 **Salvi S**, Casadio V, Conteduca V, Lolli C, Gurioli G, Martignano F, Schepisi G, Testoni S, Scarpi E, Amadori D, Calistri D, Attard G, De Giorgi U. Circulating AR copy number and outcome to enzalutamide in docetaxel-treated metastatic castration-resistant prostate cancer. *Oncotarget* 2016; **7**: 37839-37845 [PMID: 27191887 DOI: 10.18632/oncotarget.9341]

40 **Fawzy A**, Sweify KM, El-Fayoumy HM, Nofal N. Quantitative analysis of plasma cell-free DNA and its DNA integrity in patients with metastatic prostate cancer using ALU sequence. *J Egypt Natl Canc Inst* 2016; **28**: 235-242 [PMID: 27634416 DOI: 10.1016/j.jnci.2016.08.003]

41 **Azad AA**, Volik SV, Wyatt AW, Haegert A, Le Bihan S, Bell RH, Anderson SA, McConeghy B, Shukin R, Bazov J, Youngren J, Paris P, Thomas G, Small EJ, Wang Y, Gleave ME, Collins CC, Chi KN. 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 [PMID: 25712683 DOI: 10.1158/1078-0432.CCR-14-2666]

42 **Deligezer U**, Yaman F, Darendeliler E, Dizdar Y, Holdenrieder S, Kovancilar M, Dalay N. Post-treatment circulating plasma BMP6 mRNA and H3K27 methylation levels discriminate metastatic prostate cancer from localized disease. *Clin Chim Acta* 2010; **411**: 1452-1456 [PMID: 20573596 DOI: 10.1016/j.cca.2010.05.040]

43 **Schwarzenbach H**, Alix-Panabières C, Müller I, Letang N, Vendrell JP, Rebillard X, Pantel K. Cell-free

tumor DNA in blood plasma as a marker for circulating tumor cells in prostate cancer. *Clin Cancer Res* 2009; **15**: 1032-1038 [PMID: 19188176 DOI: 10.1158/1078-0432.CCR-08-1910]

44 **Bastian PJ**, Palapattu GS, Yegnasubramanian S, Lin X, Rogers CG, Mangold LA, Trock B, Eisenberger M, Partin AW, Nelson WG. Prognostic value of preoperative serum cell-free circulating DNA in men with prostate cancer undergoing radical prostatectomy. *Clin Cancer Res* 2007; **13**: 5361-5367 [PMID: 17875764 DOI: 10.1158/1078-0432.CCR-06-2781]

45 **Brindley GS**, Polkey CE, Rushton DN. Electrical splinting of the knee in paraplegia. *Paraplegia* 1979; **16**: 428-437 [PMID: 311910 DOI: 10.2147/CMAR.S192646]

46 **Khani M**, Hosseini J, Mirfakhraie R, Habibi M, Azargashb E, Pouresmaeili F. The value of the plasma circulating cell-free DNA concentration and integrity index as a clinical tool for prostate cancer diagnosis: a prospective case-control cohort study in an Iranian population. *Cancer Manag Res* 2019; **11**: 4549-4556 [PMID: 31191016 DOI: 10.2147/CMAR.S192646]

47 **Hodara E**, Morrison G, Cunha A, Zainfeld D, Xu T, Xu Y, Dempsey PW, Pagano PC, Bischoff F, Khurana A, Koo S, Ting M, Cotter PD, Moore MW, Gunn S, Usher J, Rabizadeh S, Danenberg P, Danenberg K, Carpten J, Dorff T, Quinn D, Goldkorn A. Multiparametric liquid biopsy analysis in metastatic prostate cancer. *JCI Insight* 2019; 4 [PMID: 30702443 DOI: 10.1172/jci.insight.125529]

48 **Joncas FH**, Lucien F, Rouleau M, Morin F, Leong HS, Pouliot F, Fradet Y, Gilbert C, Toren P. Plasma extracellular vesicles as phenotypic biomarkers in prostate cancer patients. *Prostate* 2019; **79**: 1767-1776 [PMID: 31475741 DOI: 10.1002/pros.23901]

49 **Mohammadi Torbati P**, Asadi F, Fard-Esfahani P. Circulating miR-20a and miR-26a as Biomarkers in Prostate Cancer. *Asian Pac J Cancer Prev* 2019; **20**: 1453-1456 [PMID: 31127907 DOI: 10.31557/APJCP.2019.20.5.1453]

50 **Ishiba T**, Hoffmann AC, Usher J, Elshimali Y, Sturdevant T, Dang M, Jaimes Y, Tyagi R, Gonzales R, Grino M, Pinski JK, Barzi A, Raez LE, Eberhardt WE, Theegarten D, Lenz HJ, Uetake H, Danenberg PV, Danenberg K. Frequencies and expression levels of programmed death ligand 1 (PD-L1) in circulating tumor RNA (ctRNA) in various cancer types. *Biochem Biophys Res Commun* 2018; **500**: 621-625 [PMID: 29679564 DOI: 10.1016/j.bbrc.2018.04.120]

51 **Wang YH**, Ji J, Wang BC, Chen H, Yang ZH, Wang K, Luo CL, Zhang WW, Wang FB, Zhang XL. Tumor-Derived Exosomal Long Noncoding RNAs as Promising Diagnostic Biomarkers for Prostate Cancer. *Cell Physiol Biochem* 2018; **46**: 532-545 [PMID: 29614511 DOI: 10.1159/000488620]

52 **Zedan AH**, Hansen TF, Assenholt J, Madsen JS, Osther PJS. Circulating miRNAs in localized/locally advanced prostate cancer patients after radical prostatectomy and radiotherapy. *Prostate* 2019; **79**: 425-432 [PMID: 30537232 DOI: 10.1002/pros.23748]

53 **Farran B**, Dyson G, Craig D, Dombkowski A, Beebe-Dimmer JL, Powell IJ, Podgorski I, Heilbrun L, Bolton S, Bock CH. A study of circulating microRNAs identifies a new potential biomarker panel to distinguish aggressive prostate cancer. *Carcinogenesis* 2018; **39**: 556-561 [PMID: 29471417 DOI: 10.1093/carcin/bgy025]

54 **Liu RSC**, Olkhov-Mitsel E, Jeyapala R, Zhao F, Commisso K, Klotz L, Loblaw A, Liu SK, Vesprini D, Fleshner NE, Bapat B. Assessment of Serum microRNA Biomarkers to Predict Reclassification of Prostate Cancer in Patients on Active Surveillance. *J Urol* 2018; **199**: 1475-1481 [PMID: 29246734 DOI: 10.1016/j.juro.2017.12.006]

55 **Albitar M**, Ma W, Lund L, Shahbaba B, Uchio E, Feddersen S, Moylan D, Wojno K, Shore N. Prostatectomy-based validation of combined urine and plasma test for predicting high grade prostate cancer. *Prostate* 2018; **78**: 294-299 [PMID: 29315679 DOI: 10.1002/pros.23473]

56 **Endzeliņš E**, Berger A, Melne V, Bajo-Santos C, Soboļevska K, Ābols A, Rodriguez M, Šantare D, Rudņickiha A, Lietuvietis V, Llorente A, Linē A. Detection of circulating miRNAs: comparative analysis of extracellular vesicle-incorporated miRNAs and cell-free miRNAs in whole plasma of prostate cancer patients. *BMC Cancer* 2017; **17**: 730 [PMID: 29121858 DOI: 10.1186/s12885-017-3737-z]

57 **McDonald AC**, Vira M, Shen J, Sanda M, Raman JD, Liao J, Patil D, Taioli E. Circulating microRNAs in plasma as potential biomarkers for the early detection of prostate cancer. *Prostate* 2018; **78**: 411-418 [PMID: 29383739 DOI: 10.1002/pros.23485]

58 **Alhasan AH**, Scott AW, Wu JJ, Feng G, Meeks JJ, Thaxton CS, Mirkin CA. Circulating microRNA signature for the diagnosis of very high-risk prostate cancer. *Proc Natl Acad Sci USA* 2016; **113**: 10655-10660 [PMID: 27601638 DOI: 10.1073/pnas.1611596113]

59 **Yan C**, Kim YH, Kang HW, Seo SP, Jeong P, Lee IS, Kim D, Kim JM, Choi YH, Moon SK, Yun SJ, Kim WJ. Urinary Nucleic Acid TSPAN13-to-S100A9 Ratio as a Diagnostic Marker in Prostate Cancer. *J Korean Med Sci* 2015; **30**: 1784-1792 [PMID: 26713053 DOI: 10.3346/jkms.2015.30.12.1784]

60 **Antonarakis ES**, Lu C, Wang H, Luber B, Nakazawa M, Roeser JC, Chen Y, Mohammad TA, Chen Y, Fedor HL, Lotan TL, Zheng Q, De Marzo AM, Isaacs JT, Isaacs WB, Nadal R, Paller CJ, Denmeade SR, Carducci MA, Eisenberger MA, Luo J. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *N Engl J Med* 2014; **371**: 1028-1038 [PMID: 25184630 DOI: 10.1056/NEJMoa1315815]

61 **Korzeniewski N**, Tosev G, Pahernik S, Hadaschik B, Hohenfellner M, Duensing S. Identification of cell-free microRNAs in the urine of patients with prostate cancer. *Urol Oncol* 2015; **33**: 16.e17-16.e22 [PMID: 25445383 DOI: 10.1016/j.urolonc.2014.09.015]

62 **Papadopoulou E**, Davilas E, Sotiriou V, Georgakopoulos E, Georgakopoulou S, Koliopanos A, Aggelakis F, Dardoufas K, Agnanti NJ, Karydas I, Nasioulas G. Cell-free DNA and RNA in plasma as a new molecular marker for prostate and breast cancer. *Ann N Y Acad Sci* 2006; **1075**: 235-243 [PMID: 17108217 DOI: 10.1196/annals.1368.032]

63 **Di Meo A**, Bartlett J, Cheng Y, Pasic MD, Yousef GM. Liquid biopsy: a step forward towards precision medicine in urologic malignancies. *Mol Cancer* 2017; **16**: 80 [PMID: 28410618 DOI: 10.1186/s12943-017-0644-5]

64 **Wadosky KM**, Koochekpour S. Molecular mechanisms underlying resistance to androgen deprivation therapy in prostate cancer. *Oncotarget* 2016; **7**: 64447-64470 [PMID: 27487144 DOI: 10.18632/oncotarget.10901]

65 **Sobhani N**, Generali D, D'Angelo A, Aieta M, Roviello G. Current status of androgen receptor-splice variant 7 inhibitor niclosamide in castrate-resistant prostate-cancer. *Invest New Drugs* 2018; **36**: 1133-1137 [PMID: 30083960 DOI: 10.1007/s10637-018-0653-2]

66 **Dehm SM**, Schmidt LJ, Heemers HV, Vessella RL, Tindall DJ. Splicing of a novel androgen receptor exon generates a constitutively active androgen receptor that mediates prostate cancer therapy resistance. *Cancer Res* 2008; **68**: 5469-5477 [PMID: 18593950 DOI: 10.1158/0008-5472.CAN-08-0594]

67 **Hu R**, Dunn TA, Wei S, Isharwal S, Veltri RW, Humphreys E, Han M, Partin AW, Vessella RL, Isaacs WB, Bova GS, Luo J. Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. *Cancer Res* 2009; **69**: 16-22 [PMID: 19117982 DOI: 10.1158/0008-5472.CAN-08-2764]

68 **Steinestel J**, Luedeke M, Arndt A, Schnoeller TJ, Lennerz JK, Wurm C, Maier C, Cronauer MV, Steinestel K, Schrader AJ. Detecting predictive androgen receptor modifications in circulating prostate cancer cells. *Oncotarget* 2019; **10**: 4213-4223 [PMID: 31289619 DOI: 10.18632/oncotarget.3925]

69 **Scher HI**, Graf RP, Schreiber NA, McLaughlin B, Lu D, Louw J, Danila DC, Dugan L, Johnson A, Heller G, Fleisher M, Dittamore R. Nuclear-specific AR-V7 Protein Localization is Necessary to Guide Treatment Selection in Metastatic Castration-resistant Prostate Cancer. *Eur Urol* 2017; **71**: 874-882 [PMID: 27979426 DOI: 10.1016/j.eururo.2016.11.024]

70 **Richardsen E**, Andersen S, Melbø-Jørgensen C, Rakaee M, Ness N, Al-Saad S, Nordby Y, Pedersen MI, Dønnem T, Bremnes RM, Busund LT. MicroRNA 141 is associated to outcome and aggressive tumor characteristics in prostate cancer. *Sci Rep* 2019; **9**: 386 [PMID: 30674952 DOI: 10.1038/s41598-018-36854-7]

71 **Giraldez MD**, Chevillet JR, Tewari M. Droplet Digital PCR for Absolute Quantification of Extracellular MicroRNAs in Plasma and Serum: Quantification of the Cancer Biomarker hsa-miR-141. *Methods Mol Biol* 2018; **1768**: 459-474 [PMID: 29717459 DOI: 10.1007/978-1-4939-7778-9_26]

72 **Ibrahim NH**, Abdellateif MS, Kassem SH, Abd El Salam MA, El Gammal MM. Diagnostic significance of miR-21, miR-141, miR-18a and miR-221 as novel biomarkers in prostate cancer among Egyptian patients. *Andrologia* 2019; **51**: e13384 [PMID: 31483058 DOI: 10.1111/and.13384]

73 **Karzai FH**, Madan RA, Figg WD. Beyond PSA: managing modern therapeutic options in metastatic castration-resistant prostate cancer. *South Med J* 2015; **108**: 224-228 [PMID: 25871992 DOI: 10.14423/SMJ.0000000000000266]

74 **Aggarwal R**, Romero GR, Friedl V, Weinstein A, Foye A, Huang J, Feng F, Stuart JM, Small EJ. Clinical and genomic characterization of Low PSA Secretors: a unique subset of metastatic castration resistant prostate cancer. *Prostate Cancer Prostatic Dis* 2020 [PMID: 32286548 DOI: 10.1038/s41391-020-0228-0]

75 **Buttigliero C**, Tucci M, Sonetto C, Vignani F, Di Stefano RF, Pisano C, Turco F, Lacidogna G, Guglielmini P, Numico G, Scagliotti GV, Di Maio M. Prognostic role of early PSA drop in castration resistant prostate cancer patients treated with abiraterone acetate or enzalutamide. *Minerva Urol Nefrol* 2020 [PMID: 32284527 DOI: 10.23736/S0393-2249.20.03708-X]

76 **Brady-Nicholls R**, Nagy JD, Gerke TA, Zhang T, Wang AZ, Zhang J, Gatenby RA, Enderling H. Prostate-specific antigen dynamics predict individual responses to intermittent androgen deprivation. *Nat Commun* 2020; **11**: 1750 [PMID: 32273504 DOI: 10.1038/s41467-020-15424-4]

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