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Review Article

Liquid biopsy to personalize treatment for metastatic prostate cancer

Marta Lopez-Valcarcel[1], Fernando Lopez-Campos[2], Juan Zafra[3], Irene Cienfuegos[4], Maria Ferri[5], Marta Barrado[6], Susana Hernando[7], Felipe Counago[8]

[1]Department of Radiation Oncology, Puerta de Hierro University Hospital, Madrid, Spain; [2]Department of Radiation Oncology, Ramon y Cajal University Hospital, Madrid, Spain; [3]Department of Radiation Oncology, Virgen de la Victoria University Hospital, Málaga, Spain; [4]Department of Urology, Virgen del Puerto Hospital, Plasencia, Cáceres, Extremadura, Spain; [5]Department of Radiation Oncology, Marques de Valdecilla University Hospital, Santander, Cantabria, Spain; [6]Department of Radiation Oncology, Navarra University Hospital, Pamplona, Navarra, Spain; [7]Department of Clinical Oncology, Fundación Alcorcon University Hospital, Alcorcón, Madrid, Spain; [8]Department of Radiation Oncology, GenesisCare Madrid Clinical Director, San Francisco de Asis and La Milagrosa Hospitals, National Chair of Research and Clinical Trials, GenesisCare, Madrid, Spain

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Abstract: Liquid biopsy is an innovative approach that provides a more complete understanding of treatment response and prognosis in monitoring metastatic prostate cancer. It complements invasive tissue biopsy and involves the assessment of various biomarkers in body fluids such as blood, semen, and urine. Liquid biopsy analyzes circulating tumor cells, extracellular vesicles, circulating tumor DNA, and the secretome. This is particularly important given the heterogeneity of prostate cancer and the need for better prognostic biomarkers. Liquid biopsy can personalize the treatment of homonosensitive and castration-resistant metastatic prostate cancer by acting as a predictive and prognostic tool. This review discusses various biomarkers, assay techniques, and potential applications in daily clinical practice, highlighting the exciting possibilities that this emerging field holds for improving patient outcomes.

Keywords: Liquid biopsy, prostate cancer, circulating tumor DNA, circulating tumor cells, extracellular vesicles, secretome

Introduction

Prostate cancer (PCa) is a complex disease that is underdiagnosed using current conventional tests such as prostate-specific antigen (PSA), prostate biopsy, and/or imaging modalities, that do not reflect tumor heterogeneity [1, 2]. In recent years, great progress has been made in understanding the complexity and variability of PCa, especially in advanced or metastatic stages [3]. Liquid biopsy (LB) represents a relevant advance in tumor characterization through a minimally invasive method that can analyze body fluids such as blood, saliva, semen, and urine (**Figure 1**). This allows the identification of biomarkers like circulating tumor cells (CTC), cell-free DNA (cfDNA), circulating tumor DNA (ctDNA), extracellular vesicles (EV), and the secretome [3, 4].

The real-time genomic, transcriptomic, and epigenomic information obtained by LB [5] allows continuous monitoring of tumor evolution, revealing dynamic changes that occur during active treatment or disease progression. It can be a useful tool for early detection of advanced disease, prognosis, treatment selection, response evaluation, and identification of resistance mechanisms or residual disease [1]. LB also overcomes certain limitations of solid biopsy, such as the lack of representation in terms of tumor heterogeneity, the low validity of the molecular characterization of tumor tissue processed at diagnosis, and the difficulty of sampling longitudinally or from multiple lesions in the case of tumor progression [6]. Nevertheless, the clinical application of LB is limited by the lack of standard protocols, the absence of prospective multicenter studies, and restric-

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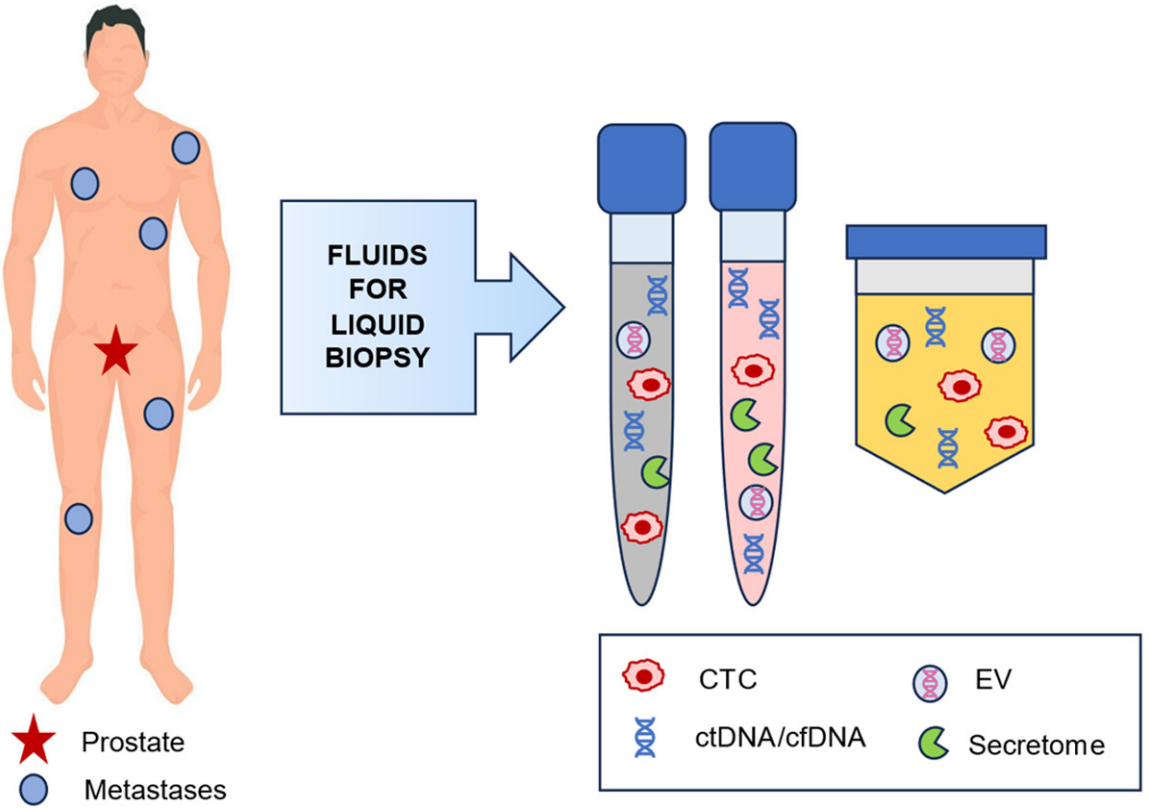

Figure 1. Fluids for liquid biopsy in metastatic prostate cancer: semen, blood, and urine. Biomarkers in liquid biopsy: CTC, ctDNA/cfDNA, EV, and secretome.

tions in terms of access and cost constraints [2, 5, 7, 8].

Materials and methods

We performed a bibliographic search in Pubmed/Medline with different keyword combinations using the formula: ("liquid biopsy" AND "metastatic prostate cancer") OR ("CTC" AND "metastatic prostate cancer") OR ("ctDNA" AND "metastatic prostate cancer") OR ("cfDNA" AND "metastatic prostate cancer") OR ("extracellular vesicles" AND "metastatic prostate cancer") OR ("secretome" AND "metastatic prostate cancer"). Through this search, we have identified the studies that informed on biomarkers for metastatic prostate cancer in LB up to October 2023.

Results

*Types of analytes in liquid biopsy (**Table 1**)*

CTC: circulating tumor cells: CTCs are an intermediate state between the primary tumor and metastases that can be evaluated in the blood as a marker of dissemination [9, 10]. Unlike PSA, CTC detection is not dependent on androgen signaling pathways [11]. CTC quantification can outperform PSA as a response biomarker, regardless of the initial number [12]. The main limitation of CTCs is their low concentration in blood (1 CTC/million leukocytes) [13]. Therefore, techniques with high sensitivity and specificity are required for sample processing, isolation, and enumeration. Currently, available methods can be divided into two groups, EpCAM (epithelial cell adhesion molecule)-dependent and EpCAM-independent [14]. One of the most widely used is CellSearch® [15], which has been approved by the Food and Drug Administration (FDA) for clinical use in patients with metastatic castration-resistant PCa (mCRPC). The study by Bono et al. reported that a favorable CTC count (< 5 cells/7.5 ml) predicted significantly better progression-free survival (PFS) and overall survival (OS) than an unfavorable count (≥ 5 cells/7.5 ml) [16].

Table 1. Liquid biopsy biomarkers in metastatic prostate cancer. Detection techniques and clinical significance of the different analytes

Biomarker	Technique	Clinical Significance
CTC 	CellSearch	CTC ≥ 5/7.5 ml and/or M-CTC > 2: worse prognosis [20]. High CTC heterogeneity: worse OS and PFS. Change in ARPIs [146]. CTC: AR-V7 positive: worse OS and PFS with ARPIs, but not with taxanes [21, 22, 24, 108]. CTC: PTEN, RB1 and TP53: aggressive variant [33, 109, 130]. CTC: BRCA2: treatment with iPARPs [101-103].
ctDNA cfDNA 	RT-qPCR NGS	High ctDNA concentration in mHSPC vs localized [33]. High ctDNA levels correlate with ADT failure [77]. High ctDNA levels: bone metastases, elevated PSA, and LDH [85, 147]. AR-V7 positivity in ctDNA: worse OS and PFS. No response to ARPIs [132]. BRCA2 and ATM in ctDNA: treatment with ARPIs [39]. TP53 mutations in CRPC on treatment with ARPIs: worse OS [38].
Extracellular Vesicles (EV) 	ddPCR RT-qPCR	Exosomal AR-V7 mRNA: worse prognosis in CRPC. Castration resistance [56]. miR-375 and miR-1290: worse OS [52]. High EV level: worse OS in CRPC [54]. CD44v8-10 mRNA copy number: resistance to docetaxel [59].
Secretome 	ELISA Proteomics	LAMC1: metastasis location and prognosis [64]. MMP 2, 3, 7, 9: disease progression and bone metastasis [68]. IL-8: invasion and metastasis [66]. IL-6: high levels in CRPC vs hormone sensitivity [65]. IL-10: high levels: resistance to ARPIs [65].

ARPIs: Androgen receptor pathway inhibitors; AR-V7: Androgen receptor splice variant 7; CTC: Circulating tumor cells; ctDNA: Circulating tumor DNA; CRPC: Castration-resistant prostate cancer; ddPCR: Droplet digital PCR; EV: Extracellular vesicles; IL: Interleukin; iPARPs: Poli (ADP-ribose) polymerase (PARP) inhibitors; LAMC1: Laminin γ1; LDH: Lactate dehydrogenase; M-CTC: mesenchymal circulating tumor cells; mHSPC: Metastatic hormone sensitive prostate cancer; miR: microRNA; MMP: Matrix metalloproteinases; mRNA: Messenger RNA; NGS: Next generation sequencing; OS: Overall survival; PFS: Progression-free survival; PSA: Prostate-specific antigen; RTq-PCR: Quantitative reverse transcriptasereal-time polymerase chain reaction.

CTCs reflect more aggressive disease after acquiring a mesenchymal phenotype during the epithelial-mesenchymal transition [17]. CTCs can be detected using the CanPatrol CTC enrichment technique, which utilizes epithelial and mesenchymal biomarkers. These cells are divided into three types: E-CTC (epithelial), M-CTC (mesenchymal), and Bi-CTC (biphenotypic: mesenchymal and epithelial) [18, 19]. A total CTC count of ≥ 5 and M-CTC ≥ 2 are independent predictors of early progression and shorter cancer-specific survival [20].

CTCs are also used as material for molecular studies. For example, in the detection of AR-V7 [21-25] and BRCA2 [26, 27], CTCs enable characterization of the aggressive variant (PTEN, RB1 and TP53) [28, 29] or sequencing to define tumor heterogeneity [28].

ctDNA and cfDNA: cfDNA is the total amount of circulating DNA in blood plasma, representing the total DNA shed by normal and tumor cells [30, 31]. It has a short half-life (from minutes to hours), which is an advantage over other protein-based biomarkers that require several weeks to assess representative changes in tumor dynamics. In addition, these changes are independent of the androgen receptor (AR) pathway [32]. Chen et al. [33] observed an increase in plasma cfDNA concentration in patients with metastatic hormone-sensitive PCa (mHSPC) compared to patients with localized PCa and healthy individuals, establishing cfDNA as a biomarker of poor prognosis.

ctDNA is the fraction of cfDNA derived from tumor cells of the primary tumor, metastases, and CTCs, that is shed into the circulation and can range from 0.01% to 90% of total cfDNA [34]. Plasma levels can vary depending on tumor type, stage, and/or tumor burden. Higher levels are observed in metastatic cancer than in localized cancer, and also correlate with progression. Tumor progression can be monitored during therapy without the limitations of intratumoral heterogeneity in solid biopsies [1]. There are several methods for the quantitative and qualitative detection of ctDNA, the two most important being digital polymerase chain

reaction (dPCR) [35], which has higher sensitivity and specificity (especially in the case of hotspot mutations), and next-generation sequencing (NGS). The latter covers a larger number of mutations and allows whole genome sequencing [36, 37].

Characterization of genomic alterations from ctDNA can identify mutations (BRCA2, ATM, TP53) [38, 39], copy number alterations [40], and structural rearrangements, which are useful as predictive and prognostic biomarkers [3]. Variations in DNA methylation, either global or locus-specific (GSTP-1, DOCK2, HALPN3, FBXO30), have been correlated with tumor burden, treatment response, and OS [6, 40-42].

Extracellular vesicles (EV): EVs are particles surrounded by a lipid bilayer. They include exosomes, microvesicles, and apoptotic bodies, all of which play a fundamental role in intercellular communication [43-45]. Through this protective layer, they can transport proteins, lipids, messenger RNA (mRNA), and microRNA (miRNA) to recipient cells, exchange genetic material, and modify the tumor microenvironment, thereby facilitating progression [46], metastasis and drug resistance [47]. They are secreted by all cell types and can reach all body fluids [48]. There is no universal method for their analysis, depending on the type of biological fluid, sample transport, and the molecule being studied. Some techniques include PCR, sequencing, western blotting, ELISA, and expression analysis [49]. Some advantages over other biomarkers are molecular stability, bioavailability, and therapeutic potential of the analysis [49]. They are some of the first biomarkers to be studied in PCa [50] and are a very active area of research. The main goal of these markers is to improve the detection of clinically significant disease and to aid in risk stratification decisions [51].

Several studies have reported changes in the expression levels of miRNAs isolated from patients with mCRPC that have prognostic value [52, 53]. It should be noted that a higher concentration of EVs in plasma correlates with worse OS and castration resistance [54]. Expression of EV AR-V7 correlates with worse PFS and OS [55-58]. The number of mRNA copies predicts resistance to docetaxel [59]. The release of BRN4 and BRN2 mRNA from serum EV may modulate the progression of neuroendocrine PCa [60].

In addition, there is emerging evidence that DNA molecules in EVs may be superior to ctDNA as a biomarker in cancer [61, 62].

Secretome: This term refers to the group of proteins secreted by tumor cells into the extracellular space (including proteinases, cytokines, and growth factors), that are involved in the processes of differentiation, invasion, metastasis, and angiogenesis [63].

The study of the metastatic PCa cell lines DU145 and PC3 identified a total of 598 secreted proteins such as laminin gamma 1 (LAMC1) and six mutated peptides capable of identifying different metastatic sites. These could help in the development of targeted therapy and become a new predictive and prognostic biomarker for metastasis [64].

IL-6 and IL-10 levels are consistently and significantly increased ($P < 0.05$) in patients with mCRPC who do not respond to abiraterone and/or enzalutamide [65]. In the CHAARTED trial, high levels of IL-8 in mHSPC before androgen deprivation therapy (ADT) predicted the development of bone metastases, castration resistance, and worse OS, independent of metastatic burden, time to metastasis, or docetaxel use ($P < 0.001$) [66]. IL-23 promotes the progression of mCRPC, so anti-IL-23 therapy could reverse castration resistance and improve the efficacy of enzalutamide [67].

Several studies have found increased levels of several metalloproteases (MMPs) such as MMP-2, -3, -7, -9, -13, -14, -15, and -26 in metastatic PCa, with a significant decrease in response to therapy [68]. Frieling et al. observed high levels of MMP-3 in patients with bone metastases [69], and Dhar et al. analyzed MMP-1, -2, -7, and -9 in mCRPC and found that clinical and biochemical responders had lower levels of MMP [70]. These results suggest that MMP could be used as a prognostic biomarker.

Cathepsins are another family of proteases that promote tumor progression. Cathepsin K expression is significantly higher in bone metas-

Table 2. Studies of liquid biopsy in mHSPC: clinical applications

Prognostic Value			
Study	Analyte	N	Result
Reichert et al. [74]	CTC count CellSearch	58	High CTC before ADT is more common in high tumor burden (P < 0.003) and correlated with no biochemical response at 7 months (P < 0.008).
Resel et al. [75]		30	CTC ≥ 4 worse OS and PFS (P < 0.001).
Yang et al. [76]	Can Patrol. M-CTC	108	> 2 M-CTC earlier development of CRPC and worse OS.
Yang et al. [20]		54	≥ 5 CTC o > 2 M-CTC worse CSS and mCRPC-free survival (P < 0.05) in oligometastatic HSPC after radical prostatectomy.
Vandekerkhove et al. [77]	ctDNA NGS	53	ctDNA is increased in de novo mHSPC, especially in visceral versus bone/nodal metastases (P < 0.03).
Agarwal et al. [78]		129	ctDNA or AR aberrations (P < 0.05), or PI3K activation are associated with worse OS (P < 0.001).
Bjerre et al. [41]	ctDNA methylation: DOCK2, HALPN3 and FBXO30 MS-ddPCR	65	ctDNA methylation increases with high burden (P < 0.001) and is associated with a shorter time to mCRPC independent of tumor burden (P = 0.012).
Cheng et al. [79]	miRNA RT-PCR	50	Baseline miR-375 levels are associated with PSA response at 28 weeks (P = 0.007).
Predictive Value			
Study	Analyte	N	Result
Goldkorn et al. [80]	CTC CellSearch	523	Undetectable CTC: almost 9 times more likely to achieve PSA ≤ 0.2 ng/ml at 7 months (P < 0.001) y 4 times more likely to achieve PFS > 2 years (P < 0.001).
Goodman et al. [81]		33	Favorable < 3 CTC/7.5 ml and unfavorable ≥ 3/7.5 ml for PSA response at 7 months (P ≤ 0.02).
Kohli et al. [82]	ctDNA PCR	139	Higher ctDNA predicted a shorter time to ADT failure (P = 0.02). ATM, BRCA1, BRCA2, and CHEK2 mutations are associated with time to ADT failure and OS.
Harshman et al. [66]	IL-8 serum	233	High levels predict shorter OS (P = 0.001) and time to CPRC (P < 0.001). IL-8 > 9.3 pg/ml on ADT: worse OS (P = 0.007).

AR: Androgen receptor; ADT: Androgen deprivation therapy; CTC: Circulating tumor cells; ctDNA: Circulating tumor DNA; mCRPC: metastatic castration-resistant prostate cancer; M-CTC: mesenchymal circulating tumor cells; MS-ddPCR: Methylation-specific droplet digital PCR; miRNA: microRNA; NGS: Next generation sequencing; RT-PCR: Reverse transcription polymerase chain reaction; PI3K: Phosphoinositide 3-kinase; mRNA: Messenger RNA; CSS: Cancer-specific survival; OS: Overall survival; PFS: Progression-free survival.

tases than in primary PCa and is absent in normal prostate tissue [71].

High levels of insulin-like growth factor 1 (IGF-1) have been associated with an increased risk of disease progression and the development of bone metastases. Ongoing clinical trials are investigating this mechanism to improve survival [72].

*mHSPC: prognostic and predictive value: (**Table 2**)*

Prognostic value: An elevated CTC count correlates with a high tumor burden (52% vs 23%; P = 0.03) [73]. High tumor burden and pre-treatment CTC are independently associated with a lack of biochemical response at seven months (P = 0.005), which may indicate a need for treatment intensification [74]. A CTC count ≥ 4 is associated with shorter OS (24 vs 45 months) and PFS (7 vs 44 months) (P < 0,001) [75]. The subgroup with mCRPC and M-CTC experience progression earlier (10.5 vs 18 months, P = 0.003) and more frequently (P = 0.013) [76]. Total CTC count ≥ 5 and M-CTC ≥ 2 are independent predictors of early progression and shorter CSS in mCRPC (P < 0.001) [20].

The plasma ctDNA fraction is increased in de novo mHSPC, especially in patients with visceral metastases, but the exposure to ADT may compromise the utility of ctDNA [77]. In the TITAN trial, the presence of ctDNA or genomic aberrations before therapy (P < 0.05) and the activation of the PIK3 pathway after apalutamide were associated with worse OS (P < 0.001) [78]. ctDNA methylation of DOCK2, HALPN3, and FBXO30 is detected in 61.5% of patients with de novo mHSPC and is significantly increased in high burden compared to low burden PCa (89.3% vs 32.1%, P < 0.001). They are also significantly associated with a shorter time to castration resistance independent of tumor burden [41].

Studies of cfDNA fragmentation have correlated the presence of larger fragments, ≥ 142-170 pb, with a more aggressive type of mHSPC. Cheng et al. found that high levels of miR-141, miR-200a, and miR-375 were significantly associated with CTC count, and miR-375 correlated with PSA response at 28 weeks (P = 0.007) [79].

Predictive value: Two-year PFS is four times higher (P < 0.001) in patients with undetectable CTCs vs ≥ 5 CTC/7.5 ml [80]. Goodman et al. identified a threshold of 3 CTC/7.5 ml as a predictor of progression in mCRPC. In addition, CTC count predicts the duration and extent of response to ADT [81].

A high ctDNA fraction is predictive of early failure to ADT (P = 0.02). Mutations in DNA repair genes (*ATM, BRCA1, BRCA2, CHEK2*) are associated with time to failure of ADT and survival in mHSPC [82].

In the CHAARTED study, high pre-ADT IL-8 predicted worse OS (P = 0.007) and shorter time to castration resistance (P < 0.001), independent of docetaxel use, metastatic burden, or metachronous vs. synchronous presentation of metastatic disease [66].

*mCRPC: prognostic and predictive value: (**Table 3**)*

Prognostic value: PSA is an imperfect biomarker of response. Up to 25% of patients experience clinical progression without an increase in PSA [83]. A baseline CTC count ≥ 5 CTC/7.5 ml correlates with worse OS (P < 0.0001) [16, 84]. The prognosis improves with decreasing CTC (from 6.8 to 21.3 months) and worsens with increasing CTC (> 26 9.3 months) [16]. Baseline LDH and CTC are independent prognostic factors for OS, in contrast to PSA levels [85, 86]. High levels of miR-1290 and miR-375 were associated with shorter OS (7.23 vs 19.3 months) [52]. In addition, PSMA-positive EVs are predominant in mCRPC [87] and correlate with worse OS [54].

TP53 aberrations are associated with poor prognosis and are observed in up to 50% of mCRPC [21, 88]. This is also the case for androgen receptor aberrations (amplification or ≥ 2 mutations), with a median PFS of 1.9 versus 4.4 months compared to a single mutation (P = 0.035) [89]. Del Re et al. reported that AR-V7-positive patients have worse PFS and OS (P < 0.001) [56].

Predictive value: CTC-based parameters such as CTC = 0 at 13 weeks [90] and CTC conversion (≥ 5 CTC/7.5 ml at baseline, ≤ 4 CTC/7.5 ml at 13 weeks) [12] are significantly superior to PSA for assessing biochemical response and detecting early progression [14].

Table 3. Studies of liquid biopsy in mCRPC: clinical applications

Prognostic Value			
Study	Analyte	N	Result
De Bono et al. [16]	CTC count. CellSearch	231	CTC ≥ 5/7.5 ml: Shorter OS. Change from unfavorable to favorable count improves prognosis.
Goldkorn et al. [84]		263	CTC < 5/7.5 ml and ≥ 5/7.5 ml correlate with OS (26 vs 13 months, respectively) in 1st line of docetaxel.
Scher et al. [85]		164	High risk of death in patients starting chemotherapy: high LDH and CTC count. A predictive model for survival at 4, 8, or 12 weeks.
Okegawa et al. [86]		57	Alkaline phosphatase and CTC count are independent prognostic factors for OS. CTC ≥ 5/7.5 ml after 3 cycles of docetaxel: worse OS.
Huang et al. [52]	Exosomal RNA sequencing with qRT-PCR	100	miR-1290 y miR-375 correlate with worse OS.
Del Re et al. [56]	Exosomal RNA for AR-V7 analysis through ddPCR	36	PFS and OS significantly worse in AR-V7 positive.
Predictive Value			
Study	Analyte	N	Result
Antonarakis et al. [21]	CTC count y AR-V7 in CTC	202	Better response to abiraterone and enzalutamide: negative CTC counts better than by CTC positive/AR-V7 negative and then CTC positive/AR-V7 positive.
Heller et al. [90]	CTC count	6081	Baseline CTC > 0/7.5 ml a CTC 13th week = 0/7.5 ml: better OS. CTC count from unfavorable to favorable.
Lorente et al. [12]	CellSearch	511	Worse OS associated with CTC increase during the first weeks of abiraterone or docetaxel.
Conteduca et al. [96]	Number of AR copies and mutations in plasmatic DNA	265	An increase in AR copy number before enzalutamide or abiraterone correlates with worse OS and PFS, independent of the prior use of taxanes.
Goodall et al. [97]	cfDNA ctDNA	49	A decrease in the allele frequency of somatic mutations in response to olaparib and > 50% decrease in cfDNA at 8 weeks independently correlate with better OS.
Torquato et al. [38]	cfDNA with NGS y number of AR copies and other 45 genes	62	High ctDNA concentration: worse PSA response, PFS, and OS in the setting of abiraterone or enzalutamide. Loss of TP53 and defects in the PIK3 pathway: worse OS.
Mehra et al. [98] PROSELICA FIRSTANA	cfDNA	571	High baseline cfDNA is associated with shorter rPFS and OS after taxanes. Decrease in cfDNA during the first 9 weeks is associated with response to taxanes.
Armstrong et al. [23]	mRNA AR-V7 CTC	118	AR-V7 correlates with shorter PFS and OS in patients treated with abiraterone or enzalutamide, independent of CTC count or clinical prognostic factors.
Scher et al. [22]	AR-V7 in CTC with Epic	142	Improved OS in high-risk AR-V7 positive patients treated with taxanes compared to patients treated with ARPIs. Improved OS in AR-V7 negative patients treated with ARPIs versus taxanes (19.8 vs 12.8 months, P = 0.05).
Annala et al. [39]	cfDNA with whole exome sequencing and deep sequencing	202	BRCA2 and ATM alterations: poor response to abiraterone or enzalutamide. Somatic mutations in TP53 are independently associated with rapid resistance.
De Laere et al. [109]	CTC count RNA sequencing for CTC-ARV cfDNA sequencing for AR	168	TP53 is a negative prognostic marker for ARPIs compared to any AR-derived biomarker.
Peter et al. [110]	WGBS	16	cfDNA methylome dynamics during abiraterone/enzalutamide treatment. Methylation during treatment correlates with a longer time to clinical progression.
Mahon et al. [111]	PCR methylation	600	Methylated GSTP1 undetectable at baseline correlates with prolonged OS (P < 0.00001), and also after 2 cycles of docetaxel (P < 0.00001).
Hendriks et al. [42]	PCR methylation	50	Hypermethylation of GSTP1 and APC correlates with worse OS (P < 0.03).

AR: androgen receptor; AR-V7: androgen receptor splice variant-7; ARPIs: Androgen receptor pathway inhibitors; cfDNA: cell-free DNA; CTC: circulating tumor cells; ctDNA: circulating tumor DNA; mCRPC: metastatic castration-resistant prostate cancer; GSTP1: glutathione S-transferase pi 1; LDH: Lactate dehydrogenase; miRNA: micro RNA; NGS: next-generation sequencing; RTq-PCR: quantitative reverse transcription polymerase chain reaction; PI3K: phosphoinositide 3-kinase; mRNA: messenger RNA; OS: overall survival; PFS: progression-free survival; rPFS: radiographic Progression-Free Survival; TP53: tumor protein 53; WGBS: Whole genome bisulfite sequencing.

A rapid decrease in ctDNA (≥ 50%) is associated with prolonged PFS and OS [91-94], while an increase at 12 weeks increases the risk of early biochemical and radiographic progression (P < 0.001) [95-97]. Higher levels of cfDNA and ctDNA at baseline (≥ 30%) are independent predictors of shorter PFS and OS (P < 0.001) [39, 91, 95-98], with ctDNA considered a biomarker of response to androgen receptor pathway inhibitors (ARPIs) [38], poly (ADP-ribose) polymerase inhibitors (PARPi) [97], and taxanes [99, 100]. Mutations in DNA repair genes of the homologous recombination (HR) pathway (BRCA1, BRCA2, ATM) are present in up to 27% of mCRPC and confer sensitivity to PARPi and platinum [101-103]. In addition, patients with mutations in mismatch repair genes (MLH1, MSH2, MSH6, PMS2), which account for approximately 2-3% of cases, respond to the PD-L1 inhibitor pembrolizumab [104, 105], providing another therapeutic option.

In patients treated with PARPi, ctDNA studies have identified a heterogeneous scenario of subclonal mutations in BRCA2 or PALB2 that restore BRCA2 function at progression, and are absent at baseline [26, 97].

Approximately half of patients have ctDNA aberrations in PIK3CA/B, PTEN, or AKT [106] which are associated with poor response to ARPIs and worse radiographic progression-free survival (rPFS) (P = 0.034) [39, 107]. Loss of RB1 (P = 0.01) and upregulation of MET (P = 0.02) in ctDNA correlate significantly with PFS [29].

AR-V7 positivity is associated with worse outcome in terms of PSA response, PFS, and OS after ARPIs (P < 0.001) [21, 23, 108], but a better response to taxanes [22, 24].

Loss of TP53 in ctDNA is predictive of worse response to ARPIs [39]. De Laere et al. stratified mCRPC according to this loss before ARPIs into two groups: poor prognosis (PFS ≤ 2.5 months) and good prognosis (PFS ≥ 14 months) [109].

Changes in methylation patterns in ctDNA and cfDNA in patients treated with ARPIs are indicative of rapid disease progression and may be associated with neuroendocrine markers that determine an aggressive pattern [110, 111]. The combination of highly methylated GSTP1 + APC at baseline predicts OS (P < 0.02) and changes after treatment may define responders [42]. Bhagirath et al. [60], showed that enzalutamide increased the release of BRN4 and BRN2 mRNA by EVs and may modulate the progression of mCRPC to neuroendocrine PCa.

Future perspectives

Several lines of research are combining genomic and transcriptomic analyses to improve the accuracy of current tests and provide a more complete genomic profile [112].

Some of these studies are using biomarkers as part of adaptive cancer therapy based on different cellular subpopulations that form PCa and have different susceptibilities to antiandrogen therapy [113]. These strategies attempt to prolong treatment efficacy by maintaining a balance between sensitive and resistant subclones.

Emerging analytes are being explored, including lipids, glucans, and the microbiome [114-117]. Microbiome studies have defined distinctive signatures depending on the cancer type, demonstrating its potential as a complementary tool to ctDNA [118].

Technological advances such as mass cytometry will allow for the visualization of protein expression specific to PCa and related to progression and treatment response (PSA, PSMA, androgen receptors, EpCAM, B-catenin) [119]. cfDNA sequencing can identify novel associations between somatic mutations and response, improving personalized therapy [120, 121]. Longitudinal cfDNA analysis can detect dynamic changes in methylation patterns that reflect tumor progression or treatment response [6, 122-124]. Multiparametric analysis using multiple LB techniques can provide a deeper understanding of the disease [125].

Numerous ongoing studies in patients with metastatic PCa (**Table 4**) are analyzing different treatment strategies based on the profiling and monitoring of various biomarkers to predict and evaluate response to therapy as well as possible resistance mechanisms [1, 51].

Table 4. Ongoing studies of liquid biopsy in metastatic prostate cancer

ClinicalTrials.gov NCT number	Status	Phase	Condition	Aim
NCT04015622 PROTRACT	Recruiting	Phase 2	mCRPC	Optimization of PCa treatment by analysis of ctDNA in mCRPC after abiraterone.
NCT04601441 CUARTET	Recruiting	Phase 4	mHSPC	To evaluate changes in genomic alterations for 73 PCa driver genes during apalutamide treatment.
NCT03228810	Completed	NA	mPCa	To detect and calculate ctDNA about metastasis-directed radiation ± surgery, and ADT.
NCT05116579	Recruiting	NA	mCRPC	To evaluate the value of personalized ctDNA monitoring for efficacy assessment and prediction during PARPi treatment.
NCT03903835 ProBio	Recruiting	Phase 3	mCRPC	To prolong progression-free survival by measuring plasma ctDNA and adjusting the treatment accordingly.
NCT03385655	Recruiting	Phase 2	mCRPC	To evaluate whether cfDNA can predict which patients are most likely to respond to therapy.
NCT03601143 PEARL	Recruiting	NA	mCRPC	Optimal liquid biopsy approach to detect AR-V7 and explore novel approaches to best predict resistance to ARSi in mCRPC.
NCT05188911 ANGELA	Recruiting	NA	mCRPC	To evaluate lesion heterogeneity and genomic alterations in mCRPC patients receiving abiraterone by incorporating dual-tracer PET/CT (PSMA and FDG) and ctDNA.
NCT05415787 PROMECI	Recruiting	NA	mPCa	To evaluate the technical feasibility of studying homologous recombination (HR) gene variants on ctDNA from patients with metastatic PCa.
NCT04188275 PRIMERA	Recruiting	NA	mCRPC	To determine of AR-V7 splice variants on circulating tumor cells and evaluate circulating levels of miRNA during systemic treatment.
NCT05885009 SOLTI-2102 (HOPE-PROSTATE)	Recruiting	NA	mPCa	To evaluate the feasibility and impact of liquid biopsy-based genomic profiling on treatment decision making in patients with metastatic prostate cancer in Spain.
NCT04581109 EPIDROP	Recruiting	NA	mPCa	To detect of viable CTCs using EPIDROP technology. Demonstrate non-inferiority of EPIDROP to the CellSearch system.
NCT04489719	Recruiting	Observational	mCRPC	To investigate the role of a DNA repair pathway in response to radium-223.

ADT: Androgen Deprivation Therapy; ARSi: Androgen receptor signalling inhibitor; AR-V7: Androgen receptor splice variant-7; CTC: circulating tumor cells; ctDNA: circulating tumour DNA; mCRPC: metastatic castration-resistant prostate cancer; mHSPC: metastatic hormone-sensitive prostate cancer; NA: Not applicable; PARPi: Poly (ADP-ribose) polymerase inhibitors; PCa: prostate cancer; mPCa: metastatic prostate cancer.

Discussion

The identification of genomic biomarkers requires the analysis of primary tumor samples or a biopsy of a *de novo* metastasis [126]. However, sequencing errors can be as high as 30-40%. In addition, these samples do not reveal the diversity of competing tumor subclones present at different disease sites.

It is accepted that CTC better reflects inter- and intratumor heterogeneity compared to conventional biopsy [127]. A baseline count of ≥ 5 CTC/7.5 ml [16] is an unfavorable prognostic factor predicting worse PFS and OS. The mesenchymal phenotype is associated with more aggressive tumors [17]: the presence of M-CTC ≥ 2 is an independent predictor of early progression and shorter CSS in mCRPC [18-20].

The detection of AR-V7 in CTC is a useful biomarker for predicting response to first- or second-line androgen receptor-targeted therapy [21, 24, 25, 128]. Molecular characterization of the aggressive variant (loss of PTEN, RB1 and TP53) in CTC correlates with worse PFS and OS, and higher genomic instability [28, 129], and patients with mutations in PTEN, TP53, RB1, AR, SPOP, MYC, and ATM have a higher risk of early progression [33, 130]. BRCA2 loss in CTCs is detected in up to 42% of cases, which is significantly higher than in tumor tissue analysis, thus improving the precision in detecting this alteration, which can be treated with PARPi in mCRPC [27, 129]. However, CTC isolation and characterization are limited and costly using current platforms. Systems such as AdnaTest allow CTCs to be captured while preserving the quality of the mRNA. This facilitates further expression studies of biomarkers including AR-V7.

High cfDNA levels are a potential biomarker of poor prognosis that may be associated with shorter OS and rPFS in patients treated with taxanes or ARPIs [98, 131-133]. A high ctDNA fraction (> 2%) is predictive of progression, castration resistance, and worse OS [134]. This may allow for patient selection for treatment intensification [135].

ctDNA analysis can assess genomic alterations involved in the development of metastases. The most common somatic mutations are TP53, APC, and androgen receptors. The most common amplifications are androgen receptors and MYC. These are associated with worse OS and metastasis-free survival ($P < 0.01$) [99, 136, 137]. In addition, DNA repair genes (BRCA1/2, ATM) are involved in resistance to ADT [138], early progression [139], and response to PARPi [103, 140].

Finally, EVs actively regulate phenotypic changes including metabolism [141], proliferation [142], invasiveness/metastasis [143], stromal reprogramming [144] and treatment resistance [145]. Current procedures for EV isolation, storage, processing, and characterization need to be optimized and standardized for clinical applications [43]. The number of EVs has been associated with poor OS [54]. Furthermore, high levels of miR-1290 and miR-375 correlate with poorer OS and early recurrence [52, 53]. Of note, enzalutamide increased the release of BRN4 and BRN2 mRNA from EVs and may modulate plasticity [60].

Conclusions

Liquid biopsy complements solid biopsy in the diagnosis and management of advanced PCa. The wide variety of definitions and platforms for analysis, as well as the cost and access to the necessary technology, limit the use of these biomarkers as routine tests. Many ongoing studies in this area will help to explain tumor heterogeneity and improve the clinical management of these patients.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Marta Lopez-Valcarcel, Department of Radiation Oncology, Puerta de Hierro Majadahonda University Hospital, C/Joaquin Rodrigo, 1, Majadahonda, Madrid 28222, Spain. Tel: +34-606824696; +34-911916629; E-mail: mlvalcarcel@salud.madrid.org

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