Utility and Clinical Application of Circulating Tumor DNA (ctDNA) in Advanced Prostate Cancer

Louise Kostos,1,2 Heidi Fettke,2,3 Edmond M. Kwan,4,5 Arun A. Azad1,2
1Department of Medical Oncology, Peter MacCallum Cancer Centre, Melbourne, Australia 2Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, Australia 3Cancer Research Division, Peter MacCallum Cancer Centre, Melbourne, Australia 4Vancouver Prostate Centre, Department of Urologic Sciences, The University of British Columbia, Vancouver, Canada 5BC Cancer, Vancouver Centre, Vancouver, Canada

Abstract
The treatment landscape for metastatic prostate cancer has undergone significant changes in recent years. The availability of next-generation imaging techniques and the emergence of novel therapies have led to earlier and more aggressive treatment approaches for patients. However, despite these advancements, drug resistance and progression to castration-resistant disease remain inevitable. Understanding the molecular landscape of advanced prostate cancer lies at the forefront of being able to deliver personalized therapies and more robustly risk-stratify patients, when combined with clinical factors. Advanced prostate cancer is characterized by inter- and intratumoral heterogeneity, posing challenges in comprehensively analyzing the genomic tumor profile using a solitary tissue sample. Additionally, the disease often manifests as bone-predominant metastatic tumors, making biopsies impractical in many cases. Moreover, archival tissue samples from a prostatectomy specimen may not accurately represent the current state of the tumor. To overcome these limitations, liquid biopsies using plasma samples have emerged as a minimally invasive surrogate approach to obtain real-time information on the genomic tumor profile. Growing evidence confirms the excellent concordance of liquid biopsies with tissue samples, making them an attractive alternative to traditional tissue biopsies. These assays can provide predictive and prognostic information that may enhance patient discussions and influence treatment decisions. This review focuses on the evolution and utility of circulating tumor-derived DNA (ctDNA) liquid biopsy assays in metastatic prostate cancer.

Background
Despite recent treatment advances, metastatic prostate cancer (mPC) continues to be a leading global cause of cancer-related death in men worldwide, with a 5-year survival rate below 30%[1–3]. The treatment landscape for advanced disease has become increasingly complex over the past decade, with the availability of multiple systemic therapies such as taxanes, androgen receptor pathway inhibitors (ARPIs), poly (ADP-ribose) polymerase inhibitors (PARPi), and targeted radioligand therapy. Each of these therapies is administered alongside androgen deprivation therapy (ADT). There is an emphasis on early treatment intensification with the introduction of these therapies as doublet and even triplet regimens for metastatic hormone-sensitive prostate cancer (mHSPC)[4,5]. While some clinical subgroups achieve a clear survival benefit from these approaches, not all patients benefit from treatment intensification. Lingering questions remain regarding the optimal timing of treatment intensification or de-intensification, the ideal duration of treatment, and the optimal sequencing of available therapies. Therefore, there is an urgent need for novel predictive and prognostic biomarkers to assist with risk stratification and inform treatment decisions.

To address this critical unmet need, it is crucial to prioritize the elucidation of the molecular landscape of advanced prostate cancer and apply it at an individual patient level. In this context, there is the continuous development of
tools for comprehensive molecular tumor profiling to guide treatment selection and sequencing. Currently, the gold standard approach for molecular biomarker assessment is analysis of tumor tissue[6]. However, collecting adequate tumor tissue in mPC, which often develops in remote and deeply located lesions and distant nodes, is not always feasible, with invasive biopsies often associated with significant procedural morbidities and low-quality samples that preclude serial, multiaxial biopsy[7–9]. Moreover, characterizing molecular changes during therapy and upon disease progression is challenging, potentially leading to the oversight of resistance-conferring or novel clinically actionable changes during therapy and upon disease progression and thereby offering valuable insights to inform treatment decisions that would otherwise be missed in a single-site metastatic biopsy. The potential clinical applications of ctDNA in mCRPC are outlined below (Figure 1).

Pretreatment ctDNA fraction and profile for prognostication

The prognostic value of pretreatment ctDNA levels has been further established in mCRPC, showing that a high ctDNA fraction is associated with shorter progression-free survival (PFS) and overall survival (OS) regardless of treatment received[10,18,47]. In a study evaluating 202 patients with mCRPC receiving first-line treatment with the ARPs enzalutamide or abiraterone acetate, a high ctDNA fraction (>30%) was associated not only with increased tumor burden (as indicated by elevated plasma levels of prostate-specific antigen [PSA], lactate dehydrogenase [LDH], and alkaline phosphatase [ALP]) but also with poor response to treatment even after adjusting for established clinical prognostic factors[48]. Similarly, a high baseline ctDNA fraction prior to taxane chemotherapy was associated with shorter radiographic PFS and OS, independent of other prognostic variables[49]. Furthermore, specific genomic abnormalities detected in ctDNA have prognostic implications for treatment outcomes. Patients treated with abiraterone acetate or enzalutamide who had baseline aberrations in tumor suppressor genes (TP53, RB1, or PTEN) exhibited worse survival outcomes compared to those who tested negative at baseline or showed undetectable levels by cycle 2 of treatment[47,48,50,51]. Therefore, a high pretreatment ctDNA fraction and the presence of tumor suppressor aberrations can facilitate informed discussions with patients regarding the development of treatment options and expected outcomes and potentially support a more aggressive approach to systemic therapy.

Longitudinal monitoring of treatment response

Traditionally, serial serum PSA measurements have been utilized to monitor response to treatment in mPC. However, PSA has limitations, as radiographic progression can occur in the absence of a PSA rise, and heavily pretreated patients with AR-independent disease may have no or low levels of PSA, making it difficult to interpret potential response challenges[52,53]. Serial ctDNA assays offer an alternative method for treatment monitoring. An early reduction in ctDNA concentration or fraction (within the first 9 weeks) has been associated with longer PFS and OS in patients with mCRPC patients treated with taxanes, ARPs, and PARP inhibitors[54]. This finding was maintained even after adjusting for known clinical risk factors. Similarly, a lack of response or persistent rise in ctDNA fraction has been associated with shorter PFS[57].

Application of ctDNA in Metastatic Castration-Resistant Prostate Cancer

Most genomic studies have been conducted in patients with metastatic castration-resistant prostate cancer (mCRPC), initially using tissue samples and more recently incorporating plasma ctDNA analysis. Liquid biopsies exhibit excellent concordance with tissue samples and provide an alternative to molecular profiling of the tumor[10,15]. As a peripheral blood sample contains ctDNA from multiple sites, this liquid biopsy approach has the added benefit of capturing inter- and intratumoral heterogeneity, thereby offering valuable insights to inform treatment decisions that would otherwise be missed in a single-site metastatic biopsy. The potential clinical applications of ctDNA in mCRPC are outlined below (Figure 1).

### Abbreviations

ADT androgen deprivation therapy
AR androgen receptor
ARPI androgen receptor pathway inhibitors
cfDNA cell-free DNA
CNVs copy number variants
ctDNA circulating tumor DNA
DDB DNA damage response and repair
HRB homologous recombination repair
ICCI immune checkpoint inhibitor
ICH immunohistochemistry
mCRPC metastatic castration-resistant prostate cancer
mHSPC metastatic hormone-sensitive prostate cancer
mPC metastatic prostate cancer
MSI microsatellite instability
NGS next-generation sequencing
OS overall survival
PARPi poly (ADP-ribose) polymerase inhibitors
PGR polymerase chain reaction
PFS progression-free survival
PSA prostate-specific antigen
SNVs single nucleotide variants
SVs structural variants
TMB tumor mutation burden

### The Current Landscape of ctDNA in Prostate Cancer

Since the initial discovery of the connection between cancer and ctDNA in 1994, the field of ctDNA analysis in oncology has rapidly expanded, with FDA-approved commercial assays and companion diagnostics becoming standard-of-practice for genomic profiling in many cancer types[13,15,17]. In 2015, Azad et al. published the earliest clinical research involving genomic analysis of plasma ctDNA in advanced prostate cancer. The authors successfully identified somatic androgen receptor (AR) point mutations and focal copy number gains using targeted next-generation sequencing (NGS) and array comparative genomic hybridization, respectively[18]. Furthermore, they reported an association between plasma-detected AR alterations and primary resistance to the ARP enzalutamide, providing evidence that ctDNA can be exploited to identify and understand contemporary biomarkers. Subsequent studies have shown that in mPC, ctDNA can be used to guide a high-fidelity, whole-tissue to whole-tumor tissue-derived DNA and is capable of not only recapitulating the somatic landscape of a tumor but also identifying clinically relevant driver alterations missed by a single-site biopsy[19–21]. Additionally, through serial sampling before and during treatment, ctDNA has the potential to monitor tumor progression, provide prognostic information, and thus dictate tailored treatment plans[22,23]. The investigation of ctDNA biomarkers to prognosticate mPC and predict response to targeted therapies has become widespread, with liquid biopsy approaches often incorporated into clinical trial design[24–27].

### Technical Considerations for ctDNA Analysis

As ctDNA gains significance in guiding precision-based care for men with mPC, a myriad of approaches and technological platforms is being employed (Table 1). This diversity in approaches which will provide the most robust data, including both sensitive and highly specific approaches capable of detecting point mutations, structural rearrangements, and genomic heterogeneity among lesions[34,35]. Therefore, a comprehensive approach capable of detecting point mutations, structural variants, copy number variants, and low-frequency subclonal somatic mutations is necessary for robust profiling of the prostate cancer genome.

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Early detection of treatment resistance

Analysis of genomic alterations in patients with mCRPC has identified both primary and acquired mutations associated with treatment resistance. With the increasing integration of ARPIs earlier on in the mPC disease course, resistance and the development of aggressive neuroendocrine prostate cancer may become more prevalent [52,58]. Therefore, it is crucial to use ctDNA biopsies to investigate markers of ARPI resistance. The presence and magnitude of AR gene amplification have been associated with shorter PFS outcomes [47,49,66].

Facilitating selection of personalized treatment

One of the most important advantages of ctDNA analysis is the ability to identify potentially actionable genomic aberrations, enabling the delivery of personalized treatment plans (Table 2). Detecting AR gene amplifications from the outset may assist clinicians in providing tailored treatment plans, potentially favoring taxane chemotherapy due to the known resistance to ARPIs [67].

With the introduction of PARPIs such as olaparib and rucaparib for patients with homologous recombination repair (HRR) gene mutations [69,70], guidelines now recommend testing all patients with mCRPC for somatic and germline pathogenic HRR aberrations, including BRCA1 and BRCA2 [70]. Typically, this testing is conducted on tissue samples, which often suffer from compromised DNA quality as they are archival pretreatment samples. Germline alterations can usually be detected through simultaneous analysis of leucocyte samples extracted from the buffy coat of peripheral blood after centrifugation. Determination of HRR status not only informs whether the patient can benefit from a PARP inhibitor but also predicts a favorable response to platinum chemotherapy [71]. It is important to note that clonal hematopoiesis of indeterminate potential involving DNA repair genes may lead to false-positive results, and therefore ctDNA samples should be accompanied by a whole blood control to exclude such variants [72].

Prostate cancers with PTEN loss are more sensitive to AKT inhibition, as demonstrated by the radiographic PFS benefit when combining the AKT inhibitor ipatasertib with abiraterone acetate for patients with mCRPC and PTEN loss identified through tumor immunohistochemistry [73]. PTEN loss is also predictive of a poor response to rucaparib while retaining sensitivity to docetaxel [74,75]. The prevalence of PTEN loss through ctDNA assay is comparable to that found in tissue, potentially eliminating the need for archival tissue or a fresh biopsy [76].

Prostate cancer is typically considered immunogenically “cold” due to minimal T-cell infiltrates failing to generate a significant peripheral antitumor response, with limited benefit from immune checkpoint inhibitor (ICI) therapy in unselected cohorts [77–79]. However, a subset of prostate cancer exhibits an immunogenic phenotype that may benefit from such therapy. Biomarkers detectable in ctDNA assays can help identify these patients and provide a rationale for treatment. A recent analysis found that patients with mCRPC and a tumor mutational burden (TMB) of greater than 10 mutations per megabase respond better to ICI therapy than chemotherapy [80]. Similarly, patients with mCRPC whose tumors harbor CDX2 mutations [81,82] and high microsatellite instability (MSI) [83,84] have shown vulnerability to ICI therapy. Both CDX2 and MSI can be detected using plasma ctDNA platforms, showing high concordance with matched tissue samples [84,85].

Somatic mutations in genes responsible for regulating the Wnt signaling pathway are found in up to 20% of patients with mCRPC [43]. Activating mutations in the Wnt pathway, such as CTNNB1, are associated with resistance to ARPI, and CTNNB1 mutations occur more frequently in mCRPC ctDNA samples that have progressed on abiraterone [85]. Despite the interest and development of several novel agents, Wnt-pathway–directed therapies are yet to be approved for clinical use.

Finally, the transition to AR-independent mPC is driven by lineage plasticity and can result in neuroendocrine differentiation. Confirming neuroendocrine features requires a repeat biopsy, which can be challenging due to tumor heterogeneity and the associated morbidity of metastatic biopsies. Neuroendocrine
prostate cancer is enriched with tumor suppressor gene alterations (such as TP53, PTEN, RB1), heralding an aggressive disease phenotype resistant to standard therapeutic approaches[89,90]. ctDNA methylation assays matched with tissue samples have shown high concordance for identifying neoendocrine features, potentially serving as a future surrogate for tissue biopsies in cases where neuroendocrine transformation is suspected.

cDNA analysis is now being integrated into clinical trials, both as a supplementary test conducted alongside treatment and, more recently, as a means of determining treatment intensification following administration of ADT[35]. Treatment intensification to decreases in the abundance of ctDNA in plasma is likewise associated with poorer survival and OS regardless of treatment received[41,19,47–49]. An early reduction in ctDNA concentration or ctDNA fraction associated with longer PFS and OS[54–57,100]. A lack of response or continued rise in ctDNA fraction has been associated with shorter PFS[41,49,57,60].

**ctDNA as a prognostic tool to guide upfront treatment intensification**

Kohli et al. demonstrated that baseline ctDNA fraction also holds prognostic value in mHSPC, with higher pretreatment ctDNA fractions predicting shorter OS. The combination of ctDNA fraction, volume of disease, and serum ALP levels was also more prognostic of survival than clinical factors alone, with low-volume metastatic disease and low ctDNA fraction associated with the longest OS[35]. A higher ctDNA fraction was also predictive of ADT failure and shorter metastasis-free survival[35,94].

Additionally, several prognostic genomic aberrations exist in mHSPC, and ctDNA analysis is a useful method for identifying them (see Table 2). The presence of tumor suppressor gene alterations in tissue samples is associated with early relapse and worse outcomes[95,96]. In plasma samples, baseline alterations in DNA damage response and repair (DDR) genes and loss-of-function alterations in TP53 are likewise associated with poorer PFS and OS[53]. Untreated mHSPC patients with somatic DDR mutations had significantly shorter OS and a shorter time to ADT failure[53], while the presence of germline DDR alterations predicted shorter time to developing castration-resistant disease[98,99]. Such findings can assist clinicians with risk stratification and deciding when to intensify upfront treatment for patients with mHSPC. Patients with poor prognostic factors present at baseline, such as a high ctDNA fraction and/or DDR or tumor suppressor alterations, may be considered for a more aggressive treatment regimen or enrolment in clinical trials. Conversely, the absence of detectable ctDNA at baseline or the absence of mRNA alterations suggests a less aggressive disease that may not require immediate treatment intensification.

**TABLE 2.** Examples of the clinical significance of specific ctDNA findings in advanced prostate cancer

<table>
<thead>
<tr>
<th>Disease setting</th>
<th>ctDNA finding</th>
<th>Clinical significance</th>
</tr>
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<tbody>
<tr>
<td>mHSPC</td>
<td>Baseline ctDNA fraction</td>
<td>• Higher pretreatment ctDNA fraction is predictive of ADT failure, shorter metastasis-free survival and OS[15,14,45]</td>
</tr>
<tr>
<td></td>
<td>Baseline tumor suppressor gene alterations</td>
<td>• Associated with early relapse and worse survival outcomes[95,96]</td>
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<tr>
<td></td>
<td></td>
<td>• Ablation therapy + ADT is less effective compared to patients without tumor suppressor gene alterations[51]</td>
</tr>
<tr>
<td>mCRPC</td>
<td>Baseline DDR alterations</td>
<td>• Somatic DDR mutation associated with shorter PFS and OS[95]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Germline DDR alterations predictive of a shorter time to development castration-resistant disease[98,99]</td>
</tr>
<tr>
<td>mHSPC</td>
<td>Baseline AR alterations</td>
<td>• Any AR aberration was associated with poor OS compared to patients without detectable AR alterations[100]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Predictive of a favorable response to ARPIs and improved survival outcomes[101,102]</td>
</tr>
<tr>
<td></td>
<td>cDNA fraction</td>
<td>• Higher cDNA fraction correlates with shorter PFS as well as OS regardless of treatment received[41,19,47–49]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• An early reduction in cDNA concentration or ctDNA fraction associated with longer PFS and OS[54–57,100]</td>
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<tr>
<td></td>
<td></td>
<td>• A lack of response or continued rise in cDNA fraction has been associated with shorter PFS[41,49,57,60].</td>
</tr>
<tr>
<td>mCRPC</td>
<td>Baseline tumor suppressor gene alterations</td>
<td>• Patients treated with ARPIs had worse survival outcomes compared to those without tumor suppressor gene alterations at baseline, or who reverted to undetectable by cycle 2 of treatment[47,48,50,51].</td>
</tr>
<tr>
<td></td>
<td>cDNA fraction</td>
<td>• The presence, as well as magnitude, of AR gene amplification, has been associated with shorter PFS as well as OS[48,59–61].</td>
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<tr>
<td></td>
<td></td>
<td>• Presence of HRR mutation/s predicts sensitivity to PARPi as well as platinum chemotherapy[11].</td>
</tr>
<tr>
<td></td>
<td>CTNNB1 mutation</td>
<td>• Wnt pathway activating mutations (such as CTNNB1) are associated with resistance to ARPI[50,87].</td>
</tr>
<tr>
<td></td>
<td>PTE1 loss</td>
<td>• Prostate cancers with PTE1 loss on IHC are more sensitive to AKT inhibitor[73]. PTE1 loss is also predictive for poor response to abiraterone acetate, while sensitivity to docetaxel is retained[47,75].</td>
</tr>
<tr>
<td></td>
<td>CK12K2 mutation, high-MSI, high TMB</td>
<td>• A TMB of &gt; 10 mutations per megabase[81, CDK12 mutations[81,82] and high MSI[83] predict sensitivity to ICI therapy.</td>
</tr>
</tbody>
</table>

AR: androgen receptor; ctDNA: circulating tumor DNA; DDR: DNA damage response and repair; CTNNB1: cathepsin B1; ICI: immunotherapy; mHSPC: metastatic hormone-sensitive prostate cancer; mCRPC: metastatic castration-resistant prostate cancer; mRNA: messenger RNA; MSI: microsatellite instability; OS: overall survival; PFS: progression-free survival; TMB: tumor mutational burden.
of poor prognostic aberrations may potentially spare the patient from unnecessary treatment toxicity. However, prospective data evaluating ctDNA as a prognostic tool to guide treatment decisions in mHSPC (alongside clinical parameters) is needed before ctDNA can be adopted into mainstream practice.

Genomic aberrations to guide the choice of systemic therapy in metastatic hormone-sensitive prostate cancer

Baseline tumor suppressor gene alterations are associated with worse outcomes with ARPIs in mHSPC[97]. Furthermore, the phase 3 TTitan trial, where patients received apalutamide or placebo in addition to ADT for mHSPC, found that any AR aberration combined with detectable ctDNA at baseline is associated with poor OS[100]. In such cases, the addition of docetaxel to the regimen may be particularly important. Conversely, an SPOP mutation, which occurs in approximately 5% of patients with mHSPC, predicts a favorable response to ARPs and improved survival outcomes[101].

Challenges and Limitations of ctDNA Profiling

One significant limitation of ctDNA profiling in prostate cancer is the variability in ctDNA shed into the plasma, potentially resulting in undetectable plasma tumor content. Unfortunately, up to half of mPC patients have low plasma tumor fraction (< 20%), and the dynamics of ctDNA release mechanisms and relative contributions to low plasma tumor fraction. Incorporating ctDNA with current sequencing are required to overcome the limitations of approaches such as methylation or tumor-informed clinical parameters) is needed before ctDNA can be adopted into mainstream practice.

Conclusion

The increasing complexity of optimal treatment selection and sequencing in mPC is compounded by the integration of multiple novel therapies. Clinicians urgently need the ability to molecularly profile patients to gain predictive and prognostic insights that will guide treatment decisions. The high concordance between ctDNA and tumor tissue samples, combined with its minimally invasive and easily accessible nature, makes ctDNA a highly attractive alternative to tissue biopsy for assessing a tumor’s molecular profile. By employing serial sampling, ctDNA can capture clonal heterogeneity across metastatic sites and track lineage plasticity as it develops, enabling early detection of resistant clones before they manifest clinically. However, before widespread adoption of ctDNA can be realized, several clinical limitations must be addressed. These include improving the sensitivity of analysis techniques to detect aberrations at low allele frequencies and standardizing variant interpretation pipelines. Furthermore, extensive clinical validation with large sample sizes and eventual clinical subsidization are prerequisites for the broad use of ctDNA in clinical practice.

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