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

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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

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Competing Interests

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Abbreviations

ADT androgen deprivation therapy
AR androgen receptor
ARPI androgen receptor pathway inhibitor
cfDNA cell-free DNA
CNVs copy number variants
ctDNA circulating tumor DNA
DDR DNA damage response and repair
HRR homologous recombination repair
ICI immune checkpoint inhibitor
IHC 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
PCR polymerase chain reaction
PFS progression-free survival
PSA prostate-specific antigen
SNVs single nucleotide variants
SVs structural variants
TMB tumor mutational burden

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 with bone lesions and deep abdominal lymph nodes, is not always feasible, with invasive biopsies often associated with significant procedural morbidities and low-quality samples that preclude serial, multisite biopsies[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 clones[10]. This is especially important considering that the lethal clone involved in metastatic dissemination may not originate from the dominant foci of the primary prostate tumor[11].

Liquid biopsy approaches to molecular tumor characterization have gained attention as attractive surrogates for tumor biopsy in advanced prostate cancer over the past decade. Liquid biopsies commonly detect biomarkers such as circulating tumor cells (CTCs), cell-free DNA (cfDNA) or RNA (cfRNA), proteins, and extracellular vesicles[12]. Among these, plasma cfDNA has garnered the most interest because of its ease of sampling and established isolation and preparation protocols. The proportion of tumor-derived cfDNA is referred to as circulating tumor DNA (ctDNA), and although it is found in all fluid compartments of the body, it is best characterized from plasma. Furthermore, its short half-life (minutes to hours) and the ability to simultaneously profile both local and distant sites make it an ideal substrate for providing a comprehensive "snapshot" of the tumor[13–15].

The Current Landscape of ctDNA in Prostate Cancer

Since the initial discovery of the connection between cancer and cfDNA 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[13,16,17]. 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-detectable AR alterations and primary resistance to the ARPI enzalutamide, providing evidence that ctDNA can be exploited to identify and understand contemporary biomarkers. Subsequent studies have shown that in mPC, ctDNA is a high-fidelity substitute for solid 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 metastatic biopsy[10,19–21]. 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 collection 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**). Understanding which approach will provide the most robust data for a particular research question is crucial to translating ctDNA assays into the clinic. Advanced prostate cancer can be detected in 60% to 90% of patient plasma samples, with the ctDNA fraction varying widely among patients[10,21,28]. Consequently, high assay sensitivity is essential to avoid excluding patients from data analysis and minimize false-negative results that may compromise biomarker identification. Currently, ctDNA analysis techniques can be broadly categorized as candidate gene approaches (for < 10 loci) and high-throughput approaches[13]. Low-throughput candidate gene approaches such as digital droplet polymerase chain reaction (PCR) assays, have the highest sensitivity, enabling the detection of somatic mutations below 0.002% allelic frequency[29,30]. These approaches are valuable for monitoring treatment resistance and minimal residual disease when the targets are already known. High-throughput techniques such as NGS provide an unbiased approach to genomic analysis and are the preferred method for identifying mechanisms of treatment resistance and novel genomic biomarkers[31]. However, they are typically less sensitive and more expensive than candidate gene approaches. Recent advances in NGS technology, however, such as the inclusion of molecular barcoding, patient-specific custom panels, and significant cost reductions for short-read sequencing have enabled the detection of somatic alterations below 0.5% allelic frequency[32]. These improvements also allow for the detection of focal copy number abnormalities, which are crucial for examining the landscape of mPC. Previously, the prostate cancer genome was thought to be associated with few focal chromosomal gains or losses, but it is now clear that focal copy number alterations, such as focal deletions in *PTEN* or focal AR amplifications, play an integral role in tumor evolution and disease progression[33].

In addition to these pre-analytical assay decisions, the selection and design of the bioinformatics workflow used to profile ctDNA are crucial. mPC is typically characterized by high levels of copy number abnormalities, 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.

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 cfDNA analysis. Liquid biopsies exhibit excellent concordance with tissue samples and represent an attractive 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**).

Pretreatment ctDNA fraction and profile for prognostication

The prognostic value of pretreatment ctDNA levels has been firmly established in mCRPC, showing that higher 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 ARPIs 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 about their 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 used to monitor response to treatment in mCRPC. 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 interpretation of potential response challenging[52,53]. Serial ctDNA assays offer an alternative method for treatment monitoring. An early reduction in cfDNA concentration or fraction (within the first 9 weeks) has been associated with longer PFS and OS in patients with mCRPC patients treated with taxanes, ARPIs, and PARP inhibitors[54–57]. 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].

TABLE 1. General comparison of ctDNA analysis platforms used in advanced prostate cancer

Approach	Detection method	Genomic elements tested	Limit of detection (VAF)	Variant types detected	Estimated turnaround time	Cost		Volume of plasma required	Strengths	Limitations	Clinical uses	References
High-throughput	WGS	Entire genome	5%–10%	SNVs, indels, CNVs, SVs, fusions, rearrangements	4–6 weeks	High		10–20 mL	Provides comprehensive genomic information, no prior knowledge of loci required	High cost, time-consuming, requires complex bioinformatic analysis	Comprehensive genomic profiling, identification of rare or novel alterations	[36–39]
	WES	Exons	5%–10%		3–4 weeks	Moderate		10–20 mL	Focuses on protein-coding regions of the genome, no prior knowledge of gene required	High cost, time-consuming, misses noncoding regions, requires bioinformatic analysis	Identification of novel targets and mechanisms of treatment resistance	[39–42]
	Targeted NGS	Panel of genes/regions	1%–5%		2–3 weeks	Moderate		5–10 mL	Cost-effective and allows focused analysis, high sensitivity	High-cost, requires bioinformatic analysis, limited to selected targets, some methods cannot detect CNVs or SVs	Targeted profiling, and monitoring of known alterations, i.e., MRD detection	[39,42–44]
Candidate gene	Digital PCR	Specific mutations	≤ 0.01%	SNVs, indels, known mutations	1–2 weeks	Low		1–5 mL	Cost-effective, highly sensitive and precise detection of mutations	Limited to known mutations and loci	Detection of specific mutations for treatment allocation, monitoring treatment response	[39,45,46]
	Conventional PCR	Specific genes/regions	< 1%–5%		1–2 weeks	Low		1–5 mL	Cost-effective and allows targeted analysis, no bioinformatic analysis required	Limited to known targets, most methods only detect SNVs	Targeted mutation analysis	[39]

CNVs: copy number variants; ctDNA: circulating tumor DNA; indel: insertion/deletion; MRD: minimal residual disease; NGS: next-generation sequencing; PCR: polymerase chain reaction; SNVs: single nucleotide variants; SVs: structural variants; VAF: variant allele frequency; WES: whole exome sequencing; WGS: whole genome sequencing.

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 and OS[48,59–61]. Some AR short variants are more frequently detected in liquid biopsy samples than in tissue biopsies, making ctDNA an ideal tool for early detection of treatment-resistant clones[21]. This discordance between plasma and tissue is likely due to intratumoral heterogeneity in AR gene expression[62] and the ability of liquid biopsies to integrate genomic information from multiple metastatic sites. ctDNA may also be used to predict resistance to PARPi by detecting acquired *BRCA* reversion mutations, which are also more frequently detected in liquid biopsy samples compared to tissue and are thought to predict a poor response to PARPi[21,63]. However, a recent analysis of patients with *BRCA*-mutant mCRPC enrolled in the TRITON2 trial suggests this may not be the case, as patients who developed a *BRCA* reversion mutation while receiving rucaparib experienced better treatment outcomes[64]. In addition to detecting specific genomic aberrations, dynamic changes in ctDNA levels during therapy are also valuable for early detection of a lack of treatment response or the development of progressive disease. Early reductions in plasma ctDNA levels from baseline have been observed in patients prior to clinical determinants of treatment response[65], while persistent detectable ctDNA have been associated with worse 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 PARPi such as olaparib and rucaparib for patients with homologous recombination repair (HRR) gene mutations[68,69], 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. However, ctDNA analysis provides an easily accessible alternative for HRR status testing, showing excellent concordance with tissue samples for HRR-related gene mutations[21,56], although this depends on tumor content (ie, ctDNA fraction) in the sample. 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 abiraterone acetate while retaining sensitivity to docetaxel[74,75]. The prevalence of *PTEN* loss through cfDNA assay is comparable to that found in tissue, potentially eliminating the need for archival tissue or a fresh biopsy[76].

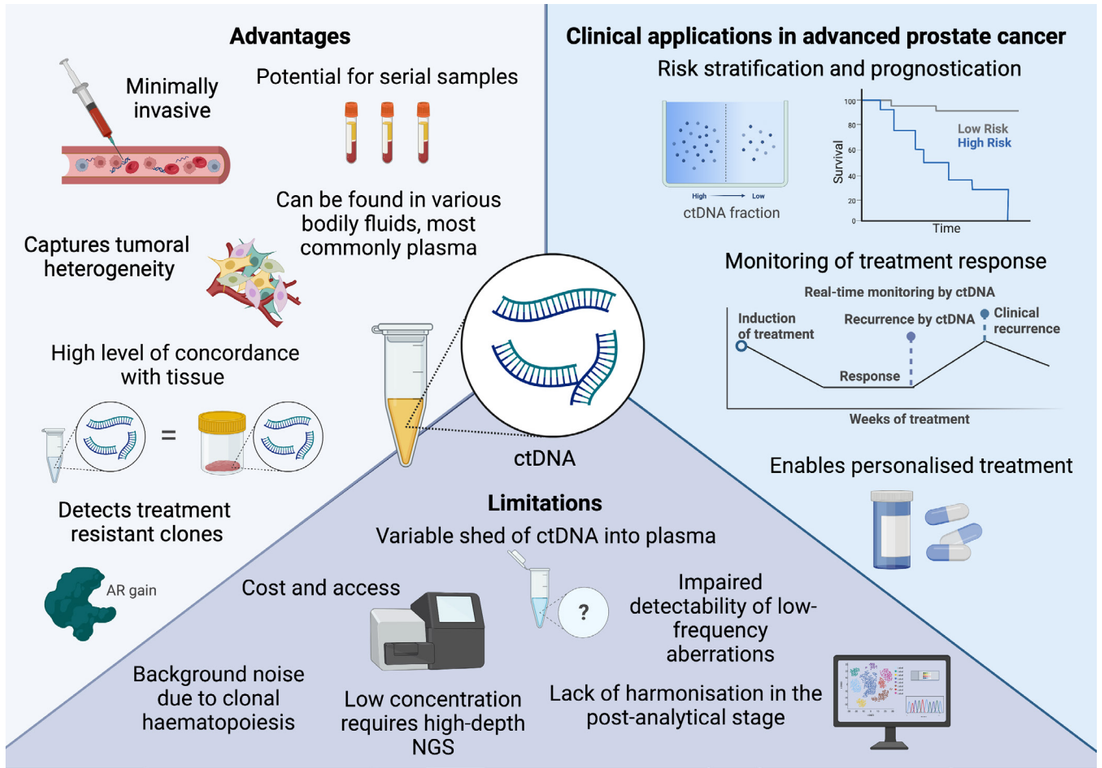
Prostate cancer is typically considered immunogenically "cold" due to minimal T-cell infiltrates failing to generate a significant peripheral antitumor response, with limited benefit from immune checkpoint inhibitor (ICI) therapy in unselected cohorts[77–79]. However, a subset of prostate cancer exhibits an immunogenic phenotype that may benefit from such therapy. Biomarkers detectable in cfDNA 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 *CDK12* mutations[81,82] and high microsatellite instability (MSI)[83] have shown vulnerability to ICI therapy. Both *CDK12* mutations 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[43,86]. Activating mutations in the Wnt pathway, such as *CTNNB1*, are associated with resistance to ARPI, and *CTNNB1* mutations occur more frequently in mCRPC cfDNA samples that have progressed on enzalutamide[50,87]. Consequently, the Wnt pathway has become an attractive target for therapeutic intervention, leading to extensive preclinical research into Wnt pathway inhibitors[88]. 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

FIGURE 1.

Advantages, limitations, and clinical applications of ctDNA in advanced prostate cancer



AR: androgen receptor; ctDNA: circulating tumor DNA; NGS: next-generation sequencing. *"Created with BioRender.com". Reproduced with permission.*

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]. cfDNA methylation assays matched with tissue samples have shown high concordance for identifying neuroendocrine features, potentially serving as a future surrogate for tissue biopsies in cases where neuroendocrine transformation is suspected.

ctDNA 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. There are two ongoing biomarker-directed clinical trials (ProBio and PC-BETS) using ctDNA analysis to guide treatment allocation in mCRPC[91–93].

Metastatic Hormone-Sensitive Prostate Cancer

The benefits of ctDNA in mHSPC are less established compared with mCRPC, primarily because of the lower cfDNA yield and ctDNA fraction observed in lower-volume, less heavily pretreated disease and due to decreases in the abundance of ctDNA in plasma following administration of ADT[35].

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

TABLE 2.

Examples of the clinical significance of specific ctDNA findings in advanced prostate cancer

Disease setting	ctDNA finding	Clinical significance
mHSPC	Baseline ctDNA fraction	• Higher pretreatment ctDNA fraction is predictive of ADT failure, shorter metastasis-free survival and OS[35,94].
	Baseline tumor suppressor gene alterations	• Associated with early relapse and worse survival outcomes[95,96]. • Abiraterone acetate + ADT less effective compared to patients without tumor suppressor gene alterations[97].
	Baseline DDR alterations	• Somatic DDR mutation associated with shorter PFS and OS[35]. • Germline DDR alterations predictive of a shorter time to developing castration-resistant disease[98,99].
	Baseline AR aberrations	• Any AR aberration was associated with poor OS compared to patients without detectable AR alterations[100].
	SPOP mutation	• Predictive of a favorable response to ARPIs and improved survival outcomes[101,102].
mCRPC	ctDNA fraction	• Higher ctDNA fraction correlates with shorter PFS as well as OS regardless of treatment received[10,13,47–49]. • An early reduction in cfDNA concentration or ctDNA fraction associated with longer PFS and OS[54–57,103]. • A lack of response or continued rise in ctDNA fraction has been associated with shorter PFS[47,49,57,66].
	Baseline tumor suppressor gene alterations	• 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].
	AR amplification	• The presence, as well as magnitude, of AR gene amplification, has been associated with shorter PFS as well as OS[48,59–61].
	HRR alterations	• Presence of HRR mutation/s predicts sensitivity to PARPi as well as platinum chemotherapy[71].
	CTNNB1 mutation	• Wnt pathway activating mutations (such as CTNNB1) are associated with resistance to ARPI[50,87].
	PTEN loss	• Prostate cancers with *PTEN* loss on IHC are more sensitive to AKT inhibition[73]. *PTEN* loss is also predictive for poor response to abiraterone acetate, while sensitivity to docetaxel is retained[74,75].
	CDK12 mutation, high-MSI, high TMB	• A TMB of > 10 mutations per megabase[80], CDK12 mutations[81,82] and high MSI[83] predict sensitivity to ICI therapy.

AR: androgen receptor; ARPI: androgen receptor pathway inhibitor; ctDNA: circulating tumor DNA; DDR: DNA damage response and repair; HRR: homologous recombination repair; IHC: Immunohistochemistry; mCRPC: metastatic castration-resistant prostate cancer; mHSPC: metastatic hormone-sensitive prostate cancer; MSI: microsatellite instability; OS: overall survival; PFS: progression-free survival; TMB: tumor mutational burden.

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[35]. Untreated mHSPC patients with somatic DDR mutations had significantly shorter OS and a shorter time to ADT failure[35], 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 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 TITAN trial, where patients received apalutamide or placebo in combination with 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 as part of triplet therapy may be particularly important. Conversely, an SPOP mutation, which occurs in approximately 5% of patients with mHSPC, predicts a favourable response to ARPIs 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 from different lesions are still not fully understood[59,76]. These samples pose challenges, as the high background signal can hinder the sensitive and specific detection of copy number variants, the identification of loss of heterozygosity (either by copy-loss or copy-neutral mechanisms), and the filtering of non-neoplastic somatic mutations arising from hematopoietic stem cells[72]. It is unlikely that ctDNA biopsies will completely replace genomic analysis of solid tissue, especially in earlier-disease stages where tumor burden is lower. Additional approaches such as methylation or tumor-informed sequencing are required to overcome the limitations of low ctDNA fraction. Incorporating ctDNA with current conventional methods will significantly advance our understanding of the biological processes underlying treatment resistance and response. Indeed, recent studies on PARP inhibitors in mCRPC have combined ctDNA analysis with tumor tissue testing to detect HRR alterations[104,105]. Continued advancements in DNA processing, sequencing technologies, and downstream bioinformatics analysis will enable the increasing integration of ctDNA into precision-oncology initiatives for mPC. However, due to the substantial infrastructural, technological, and financial requirements of ctDNA analysis, particularly for high-throughput assays, global access to ctDNA platforms at both the research and clinical levels is still limited. Furthermore, there is considerable lack of harmonization in the post-analytical stage, further complicating the implementation of ctDNA assays into the clinic (**Figure 1**). Significant efforts are still required to establish best practices for variant interpretation and reporting[106,107]. These considerations must be addressed before widespread clinical implementation of ctDNA profiling in mPC can occur.

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 limitations must be addressed. These include improving the sensitivity of analysis techniques to detect aberrations at low allele frequencies and streamlining variant interpretation pipelines. Furthermore, extensive clinical validation with large sample sizes and eventual cost subsidization are prerequisites for the broad use of ctDNA in clinical practice.

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