Circulating Tumor DNA Assessment for Treatment Monitoring Adds Value to PSA in Metastatic Castration-Resistant Prostate Cancer

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ABSTRACT

Purpose: Enzalutamide after abiraterone progression is commonly used in metastatic castration-resistant prostate cancer despite a low rate of clinical benefit. Analyzing IMbassador250, a phase III trial assessing enzalutamide with or without atezolizumab after abiraterone, we hypothesized that baseline and early changes in circulating tumor DNA (ctDNA) tumor fraction (TF) may identify patients more likely to exhibit survival benefit from enzalutamide.

Experimental Design: ctDNA was quantified from plasma samples using a tissue-agnostic assay without buffy coat sequencing. Baseline ctDNA TF, changes in ctDNA TF from baseline to cycle 3 day 1 (C3D1), and detection at C3D1 alone were compared with overall response rate, radiographic progression-free survival (rPFS), median OS (mOS), and 50% reduction in PSA.

Results: ctDNA TF detection at baseline and/or C3D1 was associated with shorter rPFS and OS in 494 evaluable patients. Detection of ctDNA TF at C3D1, with or without detection at cycle 1 day 1, was associated with worse rPFS and mOS than lack of detection. When ctDNA TF and PSA response at C3D1 were discordant, patients with (ctDNA TF undetected/PSA not reduced) had more favorable outcomes than (ctDNA TF detected/PSA reduced; mOS 22.1 vs. 16 months; $P < 0.001$).

Conclusions: In a large cohort of patients with metastatic castration-resistant prostate cancer receiving enzalutamide after abiraterone, we demonstrate the utility of a new tissue-agnostic assay for monitoring molecular response based on ctDNA TF detection and dynamics. ctDNA TF provides a minimally invasive, complementary biomarker to PSA testing and may refine personalized treatment approaches.

Introduction

Novel hormonal therapies such as abiraterone and enzalutamide have transformed the care of patients with advanced prostate cancer. However, patients eventually develop resistance and require novel therapeutic approaches. Like abiraterone, enzalutamide targets the androgen receptor (AR) signaling axis (1–4), and mechanisms of resistance to abiraterone often result in cross-resistance to enzalutamide. Still, following abiraterone progression, enzalutamide is the most-used regimen in the treatment of metastatic castration-resistant prostate cancer (mCRPC; ref. 5) despite the known low rate of net clinical benefit (6, 7). This may be due to a number of factors, including aversion to chemotherapy (8, 9), lack of access to infusion centers (10), or lack of training for chemotherapy use by urologists (11, 12). Furthermore, current standard-of-care imaging assessments and biomarkers for assessing response such as PSA have limitations (13). A biomarker that can accurately identify patients who are likely to have prolonged clinical benefit on an oral, chemotherapy-sparing regimen (enzalutamide) after abiraterone progression could be very useful.

Liquid biopsy for the analysis of circulating tumor DNA (ctDNA) in blood is an increasingly adopted tool with regulatory approvals for treatment selection and on-treatment monitoring for patients with advanced cancer. It is particularly well suited for those with inaccessible progressing lesions such as bone metastases (14). Additionally, one newly emerging liquid biopsy analyte measuring the abundance of ctDNA shed into the bloodstream is tumor fraction (TF). Several studies in mCRPC have now reported an association between a patient's TF and clinical features or tumor burden (2, 15–17). A recent real-world evaluation of 1,723 patients with mCRPC, metastatic breast cancer, metastatic colorectal carcinoma, or advanced non–small cell lung cancer found that those with elevated TF, defined in that study as 10% TF or greater, had worse relative overall survival (OS) independent of standard features used for prognosis in each of the respective disease areas (18). Furthermore, monitoring early ctDNA changes on therapy has been shown to inform treatment response and survival outcomes across therapeutic classes and tumor types (19–24). Utilizing a more comprehensive TF calculation, here called ctDNA TF, which is a composite of aneuploidy, variant allele frequency, canonical alterations, and sequencing read fragment lengths (18), we analyzed pre- and on treatment plasma to risk stratify patients with mCRPC and enable enhanced patient selection for intensified treatment approaches after abiraterone progression.

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Translational Relevance

Despite frequent use of enzalutamide after abiraterone in metastatic castration-resistant prostate cancer, most patients do not derive clinical benefit due to primary or rapidly acquired resistance. There is an urgent need to identify patients likely to progress rapidly. Radiographic assessment of treatment response and PSA exhibit limitations for the identification of nonresponders during early treatment. Longitudinal ctDNA monitoring has emerged as a powerful new technology for the assessment of treatment response in patients with advanced cancer. Using data from a randomized, phase III trial, we link the detection of ctDNA TF at baseline, on treatment, and TF dynamics with outcomes. We provide evidence that ctDNA TF is a clinically pragmatic complement, or even tiebreaker, to PSA and imaging, which are sometimes unreliable or conflicting. Taken together, ctDNA TF may inform treatment switches, provide risk stratification, and potentially guide clinical trials in patients with metastatic castration-resistant prostate cancer.

IMbassador250 (NCT03016312) sought to improve upon enzalutamide therapy, enrolling patients with mCRPC who had received prior abiraterone in the mCRPC setting and had either additionally received treatment with taxanes or refused chemotherapy. Enrolled patients were randomized to receive enzalutamide backbone with atezolizumab or placebo. The addition of atezolizumab to enzalutamide did not meet the primary endpoint of improved OS in unselected patients [stratified HR 1.12, 95% confidence interval (CI, 0.91–1.37); $P = 0.28$] (25). To enable tissue-agnostic ctDNA monitoring without paired peripheral blood mononuclear cell sequencing, FoundationOne Monitor was developed, which leverages the sequencing platform of FoundationOne Liquid CDx and enables quantification of, and changes in, ctDNA TF. Using the ctDNA TF results from banked plasma, we assessed the ctDNA content pre-enzalutamide and at cycle 3 day 1 (C3D1) with the hypothesis that early levels of and reductions in ctDNA TF on treatment are associated with radiographic response and represent a population enriched for the clinical benefit of enzalutamide.

Materials and Methods

Study design

Plasma was obtained and banked for research purposes from patients enrolled in IMbassador250 (25). Any patients with banked plasma at baseline (cycle 1 day 1, C1D1) were eligible for baseline analysis including the prognostic assessment of ctDNA TF pretreatment. Patients with banked plasma at C1D1 and C3D1 were eligible for ctDNA TF change analysis. Any patient with C3D1 plasma was eligible for the prognostic assessment of ctDNA TF on treatment.

Approval for this study, including a waiver of informed consent and Health Insurance Portability and Accountability Act waiver of authorization, was obtained from the Western Institutional Review Board (protocol 20152817).

FoundationOne Monitor assay and ctDNA tumor fraction algorithm

FoundationOne Monitor is a tissue-naïve ctDNA monitoring assay using hybrid capture NGS based on the assay methods described previously (17). The assay detects and quantifies the fraction of ctDNA of the total cell-free DNA present in a liquid biopsy sample as ctDNA TF, recently described elsewhere (26). It also reports clinically relevant alterations.

FoundationOne Monitor was performed on banked plasma specimens in a CLIA-certified, CAP-accredited laboratory (Foundation Medicine, Cambridge, MA).

In the FoundationOne Monitor assay, the ctDNA TF in a sample is quantified by integrating multiple distinct signals, including aneuploidy, the presence of short variants, and patterns related to the sizes of cell-free DNA fragments in the sample. When significant aneuploidy is present, a copy number model is constructed to explain the observed variations in relative coverage (as normalized using a panel-of-normals approach) as well as deflections in the allele frequencies of common heterozygous single nucleotide polymorphisms. The resulting model consists of a segmentation of the genome with an assigned integer copy number state for each segment in the tumor compartment, as well as a direct estimate of the fraction of DNA found in the tumor compartment. When significant aneuploidy is not present, the variant allele frequencies of short variants deemed very likely to be somatic are used to infer the likely TF in the sample based on the maximum variant allele frequency. Fragment information is used to limit the contribution of clonal hematopoiesis to the aneuploidy-based estimate and to help identify somatic short variants (27).

Statistical analysis and outcome measures

The primary outcome analysis for baseline ctDNA TF compared radiographic progression-free survival (rPFS; with OS as a secondary measure) of patients defined by ctDNA TF detected versus not detected. Whenever possible, biologically supportive analyses were conducted in accordance with the Bradford Hill Criteria (28) for inferring causality from observational studies.

Chi-square tests and Wilcoxon rank-sum tests were used to assess differences between cohorts of categorical and continuous variables, respectively. Records with missing values were excluded from analyses. PSA response was defined as ≥50% reduction from C1D1 to C3D1 (29).

rPFS was calculated from the initiation of treatment until either investigator-assessed radiographic progression or death, whichever came first. Patients without progression or death at the last follow-up were right censored. OS was calculated from treatment initiation to death from any cause, and patients with no record of mortality were right censored at the date of last contact. Seven patients with progression prior to C3D1 were excluded from progression-free survival analyses but included in OS analyses.

Radiographic response (overall response rate, ORR) was determined via the RECIST criteria in patients with measurable disease at baseline [281 of 408 patients (68.9%); refs. 25, 30].

Differences in time-to-event outcomes were assessed with the log-rank test and Cox proportional hazard models.

Harrell's concordance index (C-index; ref. 31) is a measure of model discriminatory performance, with values from 0.5 to 1.0, where 0.5 reflects a completely random result and 1.0 reflects perfect prediction (32). It was used as generated from the "survival" package in R (RRID:SCR_021137). Statistical significance was assessed from 95% confidence intervals.

Statistics, computation, and plotting were carried out using Python 2.7 (Python Software Foundation, Wilmington, DE, RRID:008394) packages matplotlib (RRID:SCR_008624), statsmodels (RRID:SCR_016074), and Pandas (RRID:SCR_018214) or R 4.2.1 (Posit, Boston, MA, RRID:SCR_001905) packages ggplot2 (RRID:

SCR_014601), survminer (RRID:SCR_021094), survival (RRID:SCR_021137), and tidyverse (RRID:SCR_019186).

Statement on investigation on humans

Informed consent was obtained for participation in the IMbassador250 study. Approval for this study, including a waiver of informed consent and Health Insurance Portability and Accountability Act waiver of authorization, was obtained from the Western Institutional Review Board (protocol 20152817).

Data availability

All relevant data are provided within the article and its accompanying Supplementary Material. Because of Health Insurance Portability and Accountability Act requirements, we are not consented to share individualized patient genomic data, which contains potentially identifying or sensitive patient information. Foundation Medicine is committed to collaborative data analysis, and we have well-established and widely used mechanisms by which investigators can query our core genomic database of >600,000 deidentified sequenced cancers to obtain aggregated datasets. More information and mechanisms for data access can be obtained by contacting the corresponding authors or the Foundation Medicine Data Governance Council at data.governance.council@foundationmedicine.com. For IMbassador250, qualified researchers may request access to individual patient-level data through the clinical study data request platform (https://search.vivli.org/enquiries).

Results

Characteristics of analysis cohort

The intention-to-treat population included 759 patients with mCRPC who had progressed on abiraterone, and either progressed after taxane treatment or refused chemotherapy (25). Of these, a total of 570 had C1D1 plasma available for sequencing; 494 of these specimens passed quality control and were successfully sequenced. Of these, 408 patients had C1D1 and C3D1 sequencing results suitable for bridging reanalysis, comprising the biomarker-evaluable population (BEP; Supplementary Figs. S1 and S2), but clinical characteristics were comparable (Supplementary Table S1). Within the BEP, the detection of ctDNA TF was strongly associated with a number of clinical factors, including higher PSA, abnormal hemoglobin, alkaline phosphatase, and a greater number of bone metastases and metastatic sites (all $P < 0.001$; Supplementary Table S2).

ctDNA TF and outcomes on enzalutamide after abiraterone progression

Patients with ctDNA TF detected at baseline (326 of 408, 80%) had considerably worse rPFS (median 4.6 vs. 11.4 months; HR, 2.21, 95% CI, 1.62–3.03; $P < 0.001$) and OS (median 13.6 vs. 22.5 months, HR, 3.25, 95% CI, 2.13–4.97; $P < 0.001$; Supplementary Fig. S3). The lowest ctDNA TF value quantified among BEP patients at baseline was 0.3%. A variable cut point sensitivity analysis observed robust results irrespective of the cut point chosen (Supplementary Table S3). The main analysis assumed that the addition of atezolizumab would have a negligible effect on this patient population. We additionally performed analyses restricted to patients who were randomized to receive enzalutamide plus atezolizumab or enzalutamide plus placebo, and the results were consistent with the overall analysis (Supplementary Fig. S4).

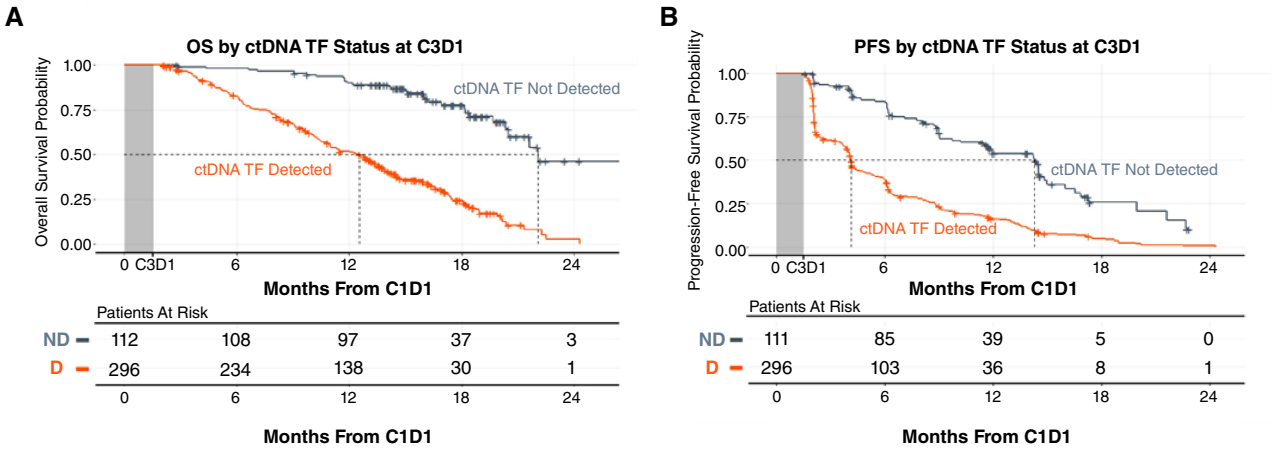
In the BEP, 296 (73%) had detectable ctDNA TF at C3D1. When evaluating ctDNA TF on treatment, detection at C3D1 was associated with shorter rPFS (HR, 3.16; 95% CI, 2.38–4.18; $P < 0.001$) and OS (HR, 5.33; 95% CI, 3.57–7.95; $P < 0.001$; **Fig. 1A** and **B**). Of patients with ctDNA TF detected at C3D1, 73% had an rPFS ≤6 months.

Given the prognostic value of ctDNA TF at both C1D1 and C3D1, we stratified patients into four groups based on the status of each timepoint (C1D1/C3D1): [Not Detected/Not Detected], [Detected/Not Detected], [Not Detected/Detected], and [Detected/Detected]. The clinical characteristics of these patients are described in Supplementary Table S4. Patients with ctDNA TF Detected at both C1D1 and C3D1 (i.e., ctDNA nonresponders, 68.6% of patients assessed) had median rPFS of 4.1 months and median OS of 12.4 months. Patients with ctDNA TF Detected at C1D1 and Not Detected at C3D1 (i.e., ctDNA responders, 11.2% of patients assessed) had a median rPFS of 14.2 months and median OS of 22.1 months. For patients having ctDNA TF Not Detected at C1D1 and Detected at C3D1 (i.e., ctDNA newly detected on treatment, 3.9% of patients assessed), median rPFS was 5.9 months, and median OS was 16.1 months, whereas those with ctDNA TF Not Detected at C1D1 and ctDNA TF Not Detected at C3D1 (i.e., ctDNA never detected, 16.2% of patients assessed) had median rPFS of 14.3 months and median OS not reached (**Fig. 2A** and **B**).

ctDNA TF and standard clinical prognostic features

The relative prognostic power was compared between baseline ctDNA TF and standard prognostic features used in metastatic prostate cancer care. In multivariable models evaluating rPFS and OS, the inclusion of baseline ctDNA TF was strongly independent in the models (Supplementary Fig. S5A and S5B). We additionally investigated the relative prognostic value of clinical factors and ctDNA TF, as well as the combined information. We calculated Harrell's concordance index (C-index) of PSA only, all clinical variables, ctDNA TF only, and clinical variables and ctDNA TF combined. The clinical variables available for analysis were largely consistent with those used in a validated nomogram for baseline prognosis in patients with chemotherapy-naïve mCRPC (33). PSA alone had the least prognostic value for both OS (0.65, Supplementary Fig. S5C) and PFS (0.56, Supplementary Fig. S5D). All clinical variables had similar prognostic values to ctDNA TF for both OS (0.71 and 0.72, respectively) and PFS (0.63 and 0.62). Clinical variables and ctDNA TF combined had a still higher OS (0.76) and PFS (0.66), indicating an independent value of ctDNA TF even when accounting for other clinical factors.

To understand the discriminatory power of response assessment modality and timing, we evaluated OS in a C-index model containing each single modality either at baseline, C3D1, or both timepoints. We investigated PSA50, ctDNA TF at C3D1 and/or C1D1, as well as RECIST assessment comparing radiographic imaging at C1D1 and C3D1. The concordance for PSA50 (0.57, SE 0.56–0.59) was outperformed by RECIST (0.62, SE 0.60–0.64; **Fig. 3**), although this difference was not statistically significant ($P > 0.05$). Concordance for ctDNA TF at baseline (0.58, SE 0.56–0.59) was largely equivalent to PSA50. Concordance for ctDNA TF at C3D1 (0.64, SE 0.63–0.65) was significantly better than baseline ctDNA TF alone ($P < 0.05$) and was similar to the combination of baseline and C3D1 ctDNA TF (0.64, SE 0.63–0.66; **Fig. 3**). The concordance of ctDNA TF at C3D1 or both timepoints when compared with OS was numerically but not significantly greater than RECIST alone. These results indicate that ctDNA TF at C3D1 is a stronger predictor of OS than 50% PSA reduction alone or ctDNA TF at C1D1 alone and slightly stronger than radiographic response, especially when combined with ctDNA TF at C1D1.



ctDNA TF at C3D1	Patients (%)	HR (95% CI)	*P*-value	Median OS (months)
Not Detected	112 (27.5%)	*Reference*	*Not Applicable*	22.1
Detected	296 (72.5%)	5.33 (3.57, 7.95)	<0.001	12.6

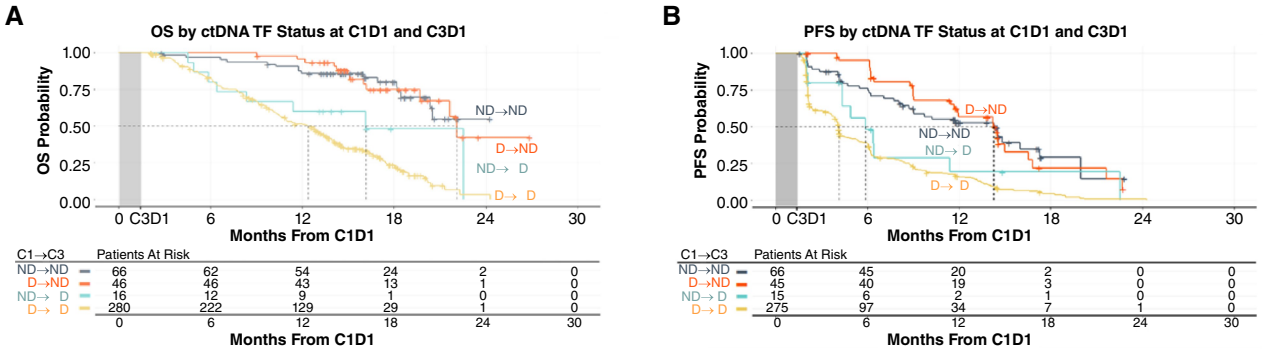
ctDNA TF at C3D1	Patients (%)	HR (95% CI)	*P*-value	Median PFS (months)
Not Detected	111 (27.7%)	*Reference*	*Not Applicable*	15.0
Detected	290 (72.3%)	3.16 (2.38, 4.18)	<0.001	4.6

Figure 1.

Lack of ctDNA TF detection at C3D1 is associated with more favorable outcomes. **A,** OS. **B,** rPFS for patients with ctDNA TF detected versus undetected at C3D1. BEP is represented. Index date is the time from initiation of therapy. Seven patients with a progression event prior to C3D1 were excluded from **B**. Gray boxes indicate the time from C1D1 to C3D1 required for inclusion in the analysis. CI, confidence interval; D, detected; HR, hazard ratio; ND, not detected.

ctDNA TF and PSA for treatment response monitoring

Change in PSA is often used as an additional response assessment in mCRPC. Comparatively, patients without a PSA reduction of at least 50% (PSA50) also had shorter rPFS (HR, 2.24; 95% CI, 1.67, 3.00) and OS (HR, 2.20; 95% CI, 1.55, 3.13; Supplementary Fig. S6). These results were consistent for PSA reduction cut points of 30% (Supplementary Fig. S7) and 90% (Supplementary Fig. S8). When evaluating the value of ctDNA TF and PSA for predicting ORR, we find that ctDNA TF at C3D1 and PSA50 response result in similar ORR predictions determined by RECIST (Supplementary Table S5).



ctDNA TF: C1D1→C3D1	Patients (%)	HR (95% CI)	*P*-value	Median OS (months)
Not Detected→ Not Detected	66 (16.2%)	*Reference*	*Not applicable*	>24 (NR)
Detected→ Not Detected	46 (11.3%)	0.99 (0.47, 2.09)	>0.9	22.1
Not Detected→ Detected	16 (3.9%)	3.19 (1.36, 7.49)	0.008	16.1
Detected→ Detected	280 (68.6%)	5.47 (3.27, 9.15)	<0.001	12.4

ctDNA TF: C1D1→C3D1	Patients (%)	HR (95% CI)	*P*-value	Median PFS (months)
Not Detected→ Not Detected	66 (16.5%)	*Reference*	*Not applicable*	14.3
Detected→ Not Detected	45 (11.2%)	0.92 (0.55, 1.52)	0.7	14.2
Not Detected→ Detected	15 (3.7%)	1.88 (0.95, 3.72)	0.069	5.9
Detected→ Detected	275 (68.6%)	3.13 (2.19, 4.47)	<0.001	4.1

Figure 2.

Combined baseline and on-treatment ctDNA TF status further stratifies patient risk. **A,** OS. **B,** rPFS for patients stratified by baseline and C3D1 ctDNA TF status. BEP is represented. Index date is the time from initiation of therapy. Seven patients with a progression event prior to C3D1 were excluded from **B**. Gray boxes indicate the time from C1D1 to C3D1 required for inclusion in the analysis. D, detected; ND, not detected; NR, not reached.

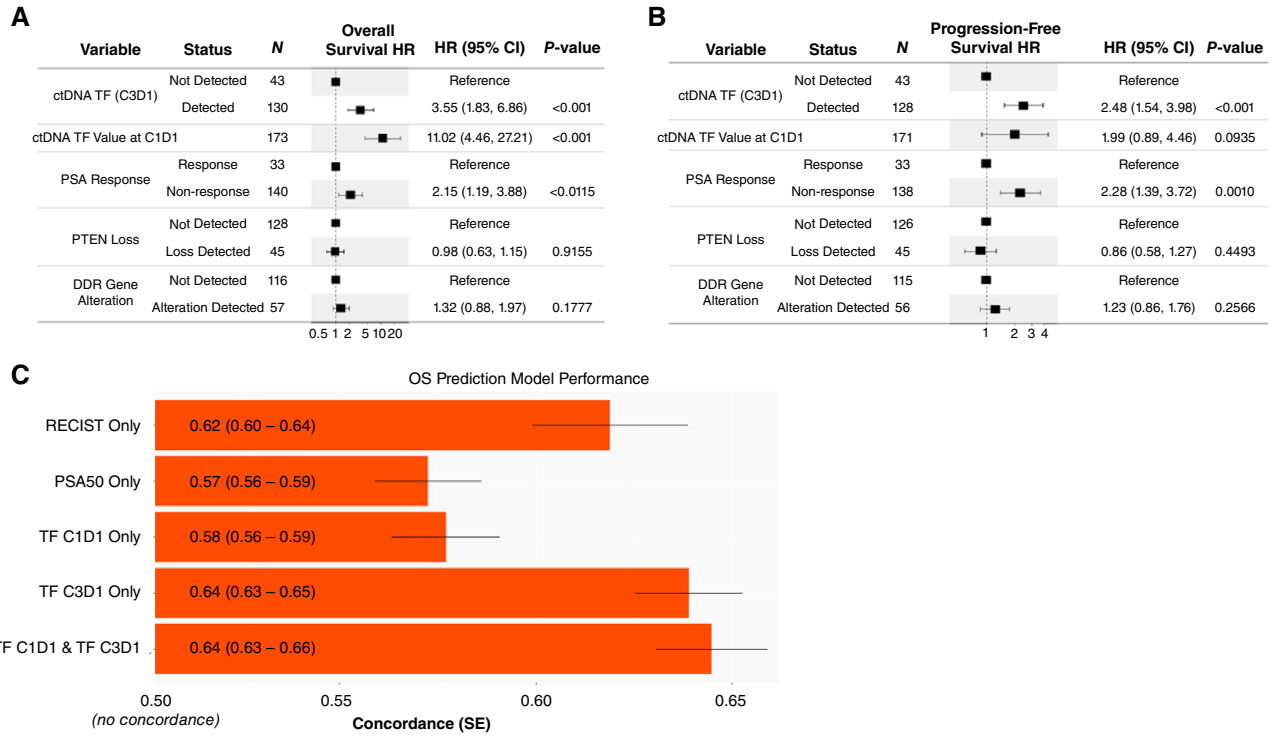


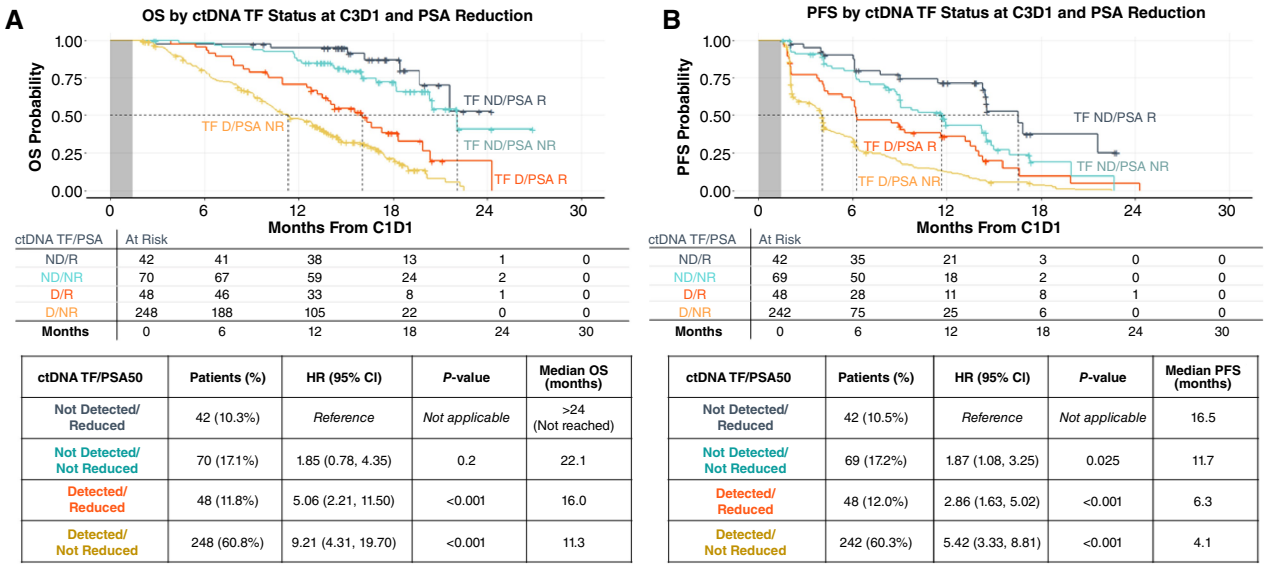
Figure 3.

Comparison of PSA, RECIST, and ctDNA TF as intermediate clinical endpoints. Multivariable model assessing ctDNA TF status at C3D1, ctDNA TF status at C1D1, PSA at C1D1, PSA response, PTEN loss, and DDR gene alterations for associations with (**A**) OS and (**B**) rPFS. **C,** C-index comparison of model performance for predicting OS. Error bars indicate standard error. TF and PSA values are categorical. "TF C1D1 and TF C3D1" includes the categorical response values at both timepoints in addition to a combination term. Axis of the C-index is displayed as starting at 0.5, consistent with the possible values of a C-index calculation.

However, only 281 of 408 patients (68.9%) were evaluable for RECIST. In clinical practice, results from different clinical laboratories and imaging can sometimes be discordant. We found that ctDNA TF detection status at C3D1 and PSA50 response at C3D1 were concordant in 71.1% of cases. To understand the relationship between PSA response and ctDNA TF detection, we conducted a survival analysis using the combination of ctDNA TF status and PSA response as a factor. When ctDNA TF status and PSA response at C3D1 were discordant, patients with [ctDNA TF Not Detected/PSA Not Reduced] had more favorable outcomes compared with [ctDNA TF Detected/PSA Reduced] (mOS 22.1 vs. 16 months; $P < 0.001$; rPFS 11.7 vs. 6.3 months) and were more similar to patients with [ctDNA TF Not Detected/PSA Reduced] (mOS Not Reduced; rPFS 16.5 months) than those with [ctDNA TF Detected/PSA Not Reduced] (mOS 16 months; rPFS 6.3 months; **Fig. 4**). Fifty out of 70 (71.4%) patients with [ctDNA TF Not Detected/PSA Not Reduced] had undetectable ctDNA TF at C1D1 as well, which correlates with good prognosis (Supplementary Fig. S2). These results were consistent when using the alternative PSA reduction cut points of 30% (Supplementary Fig. S9) and 90% (Supplementary Fig. S10). To evaluate ctDNA TF response vs. PSA50 response, we restricted the analysis to those patients with ctDNA TF detected at C1D1 and found similar results (Supplementary Fig. S11). Furthermore, when considering ctDNA TF for patients with clinically ambiguous imaging and PSA results (i.e., no radiographic progression but lack of PSA reduction), ctDNA TF detection further stratified patient risk (mOS 12.9 months vs. not reached; HR, = 4.56; $P < 0.001$; **Fig. 5**).

Discussion

Enzalutamide is the most widely used therapy after progression on abiraterone even though the clinical benefit is low due to imprecise predictors of response (5, 6). This leads to some patients receiving ineffective therapy. Within this specific clinical context, we sought to evaluate ctDNA TF, a clinically pragmatic biomarker biologically tied to both tumor growth rate and tumor burden, to identify patients most likely to benefit from enzalutamide. Utilizing reanalyzed data from a completed phase III clinical trial with an FDA-approved assay, we demonstrate that ctDNA TF detection at baseline and C3D1 on therapy are both independently prognostic for poorer outcomes on enzalutamide. Multivariable modeling suggests that consideration of both timepoints is more predictive than either timepoint alone. The data presented here suggest that the most recent ctDNA TF is the most relevant, with additional prognostic information provided by the previous timepoint(s). As has been described for other ctDNA monitoring assays in other diseases and clinical settings, the clinical performance increases with additional monitoring (20, 21, 25, 34–38). Although baseline ctDNA TF status is prognostic itself, we anticipate that each new result will enable a more up-to-date assessment that describes the current



ctDNA TF/PSA50	Patients (%)	HR (95% CI)	*P*-value	Median OS (months)
Not Detected/ Reduced	42 (10.3%)	*Reference*	*Not applicable*	>24 (Not reached)
Not Detected/ Not Reduced	70 (17.1%)	1.85 (0.78, 4.35)	0.2	22.1
Detected/ Reduced	48 (11.8%)	5.06 (2.21, 11.50)	<0.001	16.0
Detected/ Not Reduced	248 (60.8%)	9.21 (4.31, 19.70)	<0.001	11.3

ctDNA TF/PSA50	Patients (%)	HR (95% CI)	*P*-value	Median PFS (months)
Not Detected/ Reduced	42 (10.5%)	*Reference*	*Not applicable*	16.5
Not Detected/ Not Reduced	69 (17.2%)	1.87 (1.08, 3.25)	0.025	11.7
Detected/ Reduced	48 (12.0%)	2.86 (1.63, 5.02)	<0.001	6.3
Detected/ Not Reduced	242 (60.3%)	5.42 (3.33, 8.81)	<0.001	4.1

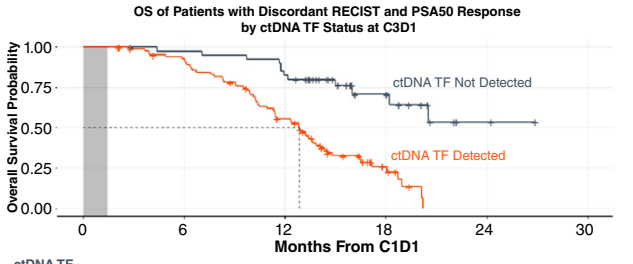
Figure 4.

ctDNA TF is more correlated with survival than PSA response in discordant cases. **A**, OS. **B,** rPFS for patients stratified by ctDNA TF and PSA response at C3D1. BEP is represented. Index date is the time from initiation of therapy. Seven patients with a progression event prior to C3D1 were excluded from **B**. TF D, ctDNA TF detected; TF ND, ctDNA TF not detected; PSA R, PSA reduced by >50%; PSA NR, PSA not reduced by >50%.

disease burden and therefore updated information to inform treatment decisions.

PSA remains readily accessible and broadly recommended by treatment guidelines, but these data indicate that evaluation of ctDNA TF on its own is more informative and can be complementary to widely used response measures such as RECIST as well as PSA at either timepoint or combined. When PSA response and ctDNA TF were discordant at C3D1, ctDNA TF status had a stronger correlation with survival outcomes. Interestingly, in instances where ctDNA TF was not detected but PSA was not reduced, baseline ctDNA TF was enriched for "not detected" (71.4% of cases), indicating a superior sensitivity of ctDNA TF in identifying low-risk patients. Furthermore, when radiographic imaging results and PSA response were discordant or inconclusive, ctDNA TF was able to risk stratify patients. This is of significant value in tumor types with high rates of bone metastasis or "bone-only" disease, such as mCRPC, where imaging is often inconclusive. Additionally, metastatic prostate cancer is particularly well suited to monitoring by ctDNA due to the relatively high prevalence of detectable aneuploidy and ctDNA shed (39). These data suggest that ctDNA TF may serve as a robust liquid biopsy analog to RECIST (40, 41). Comprehensive genomic profiling also enables the identification of novel alterations that may confer resistance to various therapies, regardless of the site of metastasis.

We acknowledge shortcomings in this analysis. Although some analyses were predefined, specifically the use of a ctDNA TF 2% cutoff for the prognostic value of ctDNA TF at C1D1 (Supplementary Table S2), and others were based on prior examples of ctDNA-based monitoring, these analyses were largely exploratory. Furthermore, although consistent with findings from across tumor types for both baseline prognosis and on-treatment monitoring, this analysis is restricted to mCRPC (18, 21, 24, 34, 35, 42–45). Most patients had RECIST-evaluable disease, although ctDNA TF and PSA results were available to evaluate the remaining 31% with RECIST-unevaluable disease. Further research in additional cohorts could evaluate the utility of ctDNA TF in patients with bone-only metastases or other clinical settings in which imaging remains challenging. Furthermore, we here analyzed on-treatment samples only from C3D1. Further exploration of optimal timing of monitoring may yield



ctDNA TF Status	Patients (%)	HR (95% CI)	*P*-value	Median OS (months)
Not Detected	41 (32.0%)	*Reference*	*Not Applicable*	>24 (not reached)
Detected	87 (68.0%)	4.56 (2.36, 8.80)	<0.001	12.9

Figure 5.

Patients with discordant imaging and PSA results can be stratified by TF. OS for patients without radiographic progression but lacking a PSA response (>50% reduction) stratified by TF response. Index date is the time from initiation of therapy. Gray box indicates the time from C1D1 to C3D1 required for inclusion in the analysis.

still better predictive value. Earlier timepoints could provide information on response earlier, potentially helping discontinue ineffective treatment sooner. Later timepoints may prove to be more strongly correlated with survival outcomes, as they would allow for more therapy exposure. In clinical practice, liquid biopsy at C3D1 would make results available for decisions about therapy discontinuation for cycle 4, complementary to imaging taken at 3 months. Subsequent research evaluating other timepoints may need to evaluate the optimal quantitative cutoff to define ctDNA response, potentially with less stringent cutoffs earlier (18, 21, 24, 34, 35, 42–45). Selection of the ideal timepoint and cutoff will require a tradeoff between clinical actionability and analytic stringency. Additionally, genomic alterations, especially in the gene encoding the AR, have been shown previously to impact prognosis on AR-directed therapies such as those examined here (46, 47). The interplay between ctDNA TF and its relation to both prognosis and sensitivity for alterations, as well as the prognostic implications for the alterations themselves, will be examined in future work.

In summary, the established methods of assessing tumor progression in mCRPC by PSA (a direct measure of prostate cancer, which is a disease mostly driven by AR) and radiographs (direct for soft tissue and indirect when assessing the cancer's impact on the bone structure such as with whole-body bone scan and CT enhanced with defined density windows to improve signal) have clear clinical utility. However, these established methods track AR-driven disease and/or require gross changes to see changes on radiographs. The data presented demonstrate how ctDNA TF complements PSA and conventional whole-body bone scan and CT imaging and are a strong prognostic factor across timepoints, making serial measurements through liquid biopsy a potentially useful tool in assessing and predicting response to therapy.

Authors' Disclosures

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Note

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References

1. Annala M, Fu S, Bacon JVW, Sipola J, Iqbal N, Ferrario C, et al. Cabazitaxel versus abiraterone or enzalutamide in poor prognosis metastatic castration-resistant prostate cancer: a multicentre, randomised, open-label, phase II trial. Ann Oncol 2021;32:896–905.
2. Annala M, Vandekerkhove G, Khalaf D, Taavitsainen S, Beja K, Warner EW, et al. Circulating tumor DNA genomics correlate with resistance to abiraterone and enzalutamide in prostate cancer. Cancer Discov 2018;8:444–57.
3. Tolmeijer SH, Boerrigter E, Schalken JA, Geerlings MJ, van Oort IM, van Erp NP, et al. A systematic review and meta-analysis on the predictive value of cell-free DNA-based androgen receptor copy number gain in patients with castration-resistant prostate cancer. JCO Precis Oncol 2020;4:714–29.
4. Wyatt AW, Azad AA, Volik SV, Annala M, Beja K, McConeghy B, et al. Genomic alterations in cell-free DNA and enzalutamide resistance in castration-resistant prostate cancer. JAMA Oncol 2016;2:1598–606.
5. George DJ, Sartor O, Miller K, Saad F, Tombal B, Kalinovský J, et al. Treatment patterns and outcomes in patients with metastatic castration-resistant prostate cancer in a real-world clinical practice setting in the United States. Clin Genitourin Cancer 2020;18:284–94.
6. de Bono JS, Chowdhury S, Feyerabend S, Elliott T, Grande E, Melhem-Bertrandt A, et al. Antitumour activity and safety of enzalutamide in patients with metastatic castration-resistant prostate cancer previously treated with abiraterone acetate plus prednisone for ≥24 weeks in europe. Eur Urol 2018;74:37–45.
7. Khalaf DJ, Annala M, Taavitsainen S, Finch DL, Oja C, Vergidis J, et al. Optimal sequencing of enzalutamide and abiraterone acetate plus prednisone in metastatic castration-resistant prostate cancer: a multicentre, randomised, open-label, phase 2, crossover trial. Lancet Oncol 2019;20:1730–9.

8. Albrecht W, Van Poppel H, Horenblas S, Mickisch G, Horwich A, Serretta V, et al. Randomized Phase II trial assessing estramustine and vinblastine combination chemotherapy vs estramustine alone in patients with progressive hormone-escaped metastatic prostate cancer. Br J Cancer 2004;90:100–5.
9. Calabrò F, Sternberg CN. Current indications for chemotherapy in prostate cancer patients. Eur Urol 2007;51:17–26.
10. Serretta V, Altieri V, Morgia G, Siragusa A, De Grande G, Napoli M, et al. Oral chemotherapy in hormone-refractory prostate carcinoma patients unwilling to be admitted to hospital. Urol Int 2024;83:452–7.
11. Crawford ED. The role of the urologist in treating patients with hormone-refractory prostate cancer. Rev Urol 2003;5(Suppl 2):S48–52.
12. Saad F. Should urologists give chemotherapy? Can Urol Assoc J 2008;2:135–6.
13. Vicini FA, Vargas C, Abner A, Kestin L, Horwitz E, Martinez A. Limitations in the use of serum prostate specific antigen levels to monitor patients after treatment for prostate cancer. Atlanta (GA): American Cancer Society; 2005.
14. Trujillo B, Wu A, Wetterskog D, Attard G. Blood-based liquid biopsies for prostate cancer: clinical opportunities and challenges. Br J Cancer 2022;127:1394–402.
15. Armstrong AJ, Luo J, Nanus DM, Giannakakou P, Szmulewitz RZ, Danila DC, et al. Prospective multicenter study of circulating tumor cell AR-V7 and taxane versus hormonal treatment outcomes in metastatic castration-resistant prostate cancer. JCO Precis Oncol 2020;4:PO.20.00200.
16. Fonseca NM, Maurice-Dror C, Herberts C, Tu W, Fan W, Murtha AJ, et al. Prediction of plasma ctDNA fraction and prognostic implications of liquid biopsy in advanced prostate cancer. Nat Commun 2024;15:1828.
17. Woodhouse R, Li M, Hughes J, Delfosse D, Skoletsky J, Ma P, et al. Clinical and analytical validation of FoundationOne liquid CDx, a novel 324-gene cfDNA-based comprehensive genomic profiling assay for cancers of solid tumor origin. PLoS One 2020;15:e0237802.
18. Reichert ZR, Morgan TM, Li G, Castellanos E, Snow T, Dall'Olio FG, et al. Prognostic value of plasma circulating tumor DNA fraction across four common cancer types: a real-world outcomes study. Ann Oncol 2023;34:111–20.
19. Cohen S, Liu M, Aleshin A. Practical recommendations for using ctDNA in clinical decision making. Nature 2023;619:259–68.
20. Lonardi S, Nimeiri H, Xu C, Zollinger DR, Madison RW, Fine AD, et al. Comprehensive genomic profiling (CGP)-informed personalized molecular residual disease (MRD) detection: an exploratory analysis from the PREDATOR study of metastatic colorectal cancer (mCRC) patients undergoing surgical resection. Int J Mol Sci 2022;23:11529.
21. Pellini B, Madison R, Childress M, Miller S, Gjoerup O, Cheng J, et al. Circulating tumor DNA monitoring on chemo-immunotherapy for risk stratification in advanced non-small cell lung cancer. Clin Cancer Res 2023;29:4596–605.
22. Tie J, Cohen J, Lahouel K, Lo S, Wang Y, Kosmider S, et al. Circulating tumor DNA analysis guiding adjuvant therapy in stage II colon cancer. N Engl J Med 2022;386:2261–72.
23. Tie J, Cohen JD, Wang Y, Christie M, Simons K, Lee M, et al. Circulating tumor DNA analyses as markers of recurrence risk and benefit of adjuvant therapy for stage III colon cancer. JAMA Oncol 2019;5:1710–7.
24. Tolmeijer SH, Boerrigter E, Sumiyoshi T, Kwan EM, Ng SWS, Annala M, et al. Early on-treatment changes in circulating tumor DNA fraction and response to enzalutamide or abiraterone in metastatic castration-resistant prostate cancer. Clin Cancer Res 2023;29:2835–44.
25. Powles T, Yuen KC, Gillessen S, Kadel EE, Rathkopf D, Matsubara N, et al. Atezolizumab with enzalutamide versus enzalutamide alone in metastatic castration-resistant prostate cancer: a randomized phase 3 trial. Nat Med 2022;28:144–53.
26. Rolfo CD, Madison RW, Pasquina LW, Brown DW, Huang Y, Hughes JD, et al. Measurement of ctDNA tumor fraction identifies informative negative liquid biopsy results and informs value of tissue confirmation. Clin Cancer Res 2024;30:2452–60.
27. Chiang A, Madison RW, Huang Y, Fine A, Jin DX, Oxnard GR, et al. Abstract 971: validation of a tumor-naïve circulating tumor DNA (ctDNA) response monitoring panel in advanced non-small cell lung cancer (aNSCLC). Cancer Res 2024;84:971.
28. Fedak KM, Bernal A, Capshaw ZA, Gross S. Applying the Bradford Hill criteria in the 21st century: how data integration has changed causal inference in molecular epidemiology. Emerg Themes Epidemiol 2015;12:14.
29. Grayling M, McMenamin M, Chandler R, Heer R, Wason J. Improving power in PSA response analyses of metastatic castration-resistant prostate cancer trials. BMC Cancer 2022;22:111.
30. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228–47.
31. Harrell FE Jr, Califf RM, Pryor DB, Lee KL, Rosati RA. Evaluating the yield of medical tests. JAMA 1982;247:2543–6.
32. Hartman N, Kim S, He K, Kalbfleisch JD. Pitfalls of the concordance index for survival outcomes. Stat Med 2023;42:2179–90.
33. Halabi S, Yang Q, Roy A, Luo B, Araujo JC, Logothetis C, et al. External validation of a prognostic model of overall survival in men with chemotherapy-naive metastatic castration-resistant prostate cancer. J Clin Oncol 2023;41:2736–46.
34. Bratman SV, Yang SYC, Iafolla MAJ, Liu Z, Hansen AR, Bedard PL, et al. Personalized circulating tumor DNA analysis as a predictive biomarker in solid tumor patients treated with pembrolizumab. Nat Cancer 2020;1:873–81.
35. Kansara M, Bhardwaj N, Thavaneswaran S, Xu C, Lee JK, Chang LB, et al. Early circulating tumor DNA dynamics as a pan-tumor biomarker for long-term clinical outcome in patients treated with durvalumab and tremelimumab. Mol Oncol 2023;17:298–311.
36. Loupakis F, Sharma S, Derouazi M, Murgioni S, Biason P, Rizzato MD, et al. Detection of molecular residual disease using personalized circulating tumor DNA assay in patients with colorectal cancer undergoing resection of metastases. JCO Precis Oncol 2021;5:PO.21.00101.
37. Powles T, Assaf ZJ, Davarpanah N, Banchereau R, Szabados BE, Yuen KC, et al. ctDNA guiding adjuvant immunotherapy in urothelial carcinoma. Nature 2021;595:432–7.
38. Powles T, Young A, Nimeiri H, Madison RW, Fine A, Zollinger DR, et al. Molecular residual disease detection in resected, muscle-invasive urothelial cancer with a tissue-based comprehensive genomic profiling-informed personalized monitoring assay. Front Oncol 2023;13:1221718.
39. Husain H, Pavlick D, Fendler B, Madison R, Decker B, Gjoerup O, et al. Tumor fraction correlates with detection of actionable variants across > 23,000 circulating tumor DNA samples. JCO Precis Oncol 2022;6:e2200261.
40. Gouda MA, Janku F, Wahida A, Buschhorn L, Schneeweiss A, Abdel Karim N, et al. Liquid biopsy response evaluation criteria in solid tumors (LB-RECIST). Ann Oncol 2024;35:267–75.
41. Jakobsen A, Spindler K. ctDNA-Response evaluation criteria in solid tumors—a new measure in medical oncology. Eur J Cancer 2023;180:180–3.
42. Collier A, Bardia A, Sohn J, Lim E, Chavez-MacGregor M, Martin M, et al. Circulating tumor DNA dynamics in acelERA Breast Cancer: a Phase II study of giredestrant for estrogen receptor-positive HER2-negative, previously treated advanced breast cancer. Cancer Res 2024;84 (Suppl 9):PO1-05-07.
43. Castellano T, Bae-Jump V, Secord AA, Danziger N, Graf R, Quintanilha JCF, et al. Liquid biopsy for advanced endometrial cancer: prognostic value of plasma circulating DNA fraction in real-world settings (1320). Gynecol Oncol 2023;176(Suppl 1):S194–5.
44. Pathak P, Li G, Quintanilha J, Keller RB, Pasquina L, Thierauf J, et al. Relationship of tumor fraction in circulating tumor DNA (ctDNA) with prognosis in patients with metastatic pancreatic cancer. J Clin Oncol 2024;42 (Suppl 3):621.
45. Miron B, Li G, Quintanilha JCF, Clark A, Scott T, Ross JS, et al. Relationship of tumor fraction in circulating tumor DNA with prognosis in patients with advanced urothelial cancer. ESMO Real World Data Digital Oncol 2024;3:100004.
46. Chandrasekar T, Yang JC, Gao AC, Evans CP. Mechanisms of resistance in castration-resistant prostate cancer (CRPC). Transl Androl Urol 2015;4:365–80.
47. Pozas J, Rodriguez SA, Fernandez VA, Burgos J, Santoni M, Manneh Kopp R, et al. Androgen receptor signaling inhibition in advanced castration resistance prostate cancer: what is expected for the near future? Cancers (Basel) 2022;14:6071.