Detection of *BRCA1*, *BRCA2*, and *ATM* Alterations in Matched Tumor Tissue and Circulating Tumor DNA in Patients with Prostate Cancer Screened in PROfound

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ABSTRACT

Purpose: Not all patients with metastatic castration-resistant prostate cancer (mCRPC) have sufficient tumor tissue available for multigene molecular testing. Furthermore, samples may fail because of difficulties within the testing procedure. Optimization of screening techniques may reduce failure rates; however, a need remains for additional testing methods to detect cancers with alterations in homologous recombination repair genes. We evaluated the utility of plasma-derived circulating tumor DNA (ctDNA) in identifying deleterious *BRCA1*, *BRCA2* (BRCA), and *ATM* alterations in screened patients with mCRPC from the phase III PROfound study.

Patients and Methods: Tumor tissue samples were sequenced prospectively at Foundation Medicine, Inc. (FMI) using an investigational next-generation sequencing (NGS) assay based on FoundationOne®CDx to inform trial eligibility. Matched ctDNA samples were retrospectively sequenced at FMI, using an investigational assay based on FoundationOne®Liquid CDx.

Results: 81% (503/619) of ctDNA samples yielded an NGS result, of which 491 had a tumor tissue result. BRCA and *ATM* status in tissue compared with ctDNA showed 81% positive percentage agreement and 92% negative percentage agreement, using tissue as reference. At variant-subtype level, using tissue as reference, concordance was high for nonsense (93%), splice (87%), and frameshift (86%) alterations but lower for large rearrangements (63%) and homozygous deletions (27%), with low ctDNA fraction being a limiting factor.

Conclusions: We demonstrate that ctDNA can greatly complement tissue testing in identifying patients with mCRPC and BRCA or *ATM* alterations who are potentially suitable for receiving targeted PARP inhibitor treatments, particularly patients with no or insufficient tissue for genomic analyses.

Introduction

Prostate cancer is a complex heterogeneous disease, and despite early treatment, up to 40% of patients develop metastases, which mostly progress to metastatic castration-resistant prostate cancer (mCRPC; ref. 1). Approximately 25% of patients with mCRPC harbor deleterious alterations in DNA damage repair genes, including those with direct or indirect roles in homologous recombination repair (HRR; refs. 2–4). The most well characterized of these are *BRCA1*, *BRCA2* (BRCA), and *ATM*. Genomic alterations that interfere with HRR have been associated with increased sensitivity to Poly (ADP-ribose) polymerase (PARP) inhibition treatment in prostate and other solid cancers (5–8).

In the PROfound trial (NCT02987543), the PARP inhibitor olaparib significantly prolonged radiographic progression-free survival (rPFS) and overall survival (OS) compared with enzalutamide or abiraterone in patients with mCRPC and tumors with alterations in BRCA and *ATM* genes (Cohort A) and who had disease progression on a prior next-generation hormonal agent (NHA; refs. 9, 10). The PROfound trial resulted in the approval of olaparib for the treatment of mCRPC in patients with qualifying HRR gene alterations who had disease progression following prior treatment with enzalutamide or abiraterone (11, 12), as well as the diagnostic approval of the FoundationOne®CDx tumor tissue assay (13, 14).

Genomic testing is a key challenge in maximizing patient access to treatments for which a genetic result is required for eligibility. Tumor tissue testing is the gold standard in identifying patients with HRR-gene-altered cancers; however, in PROfound, 31% of patients' tumor tissue samples failed molecular screening because of pathology review failure (i.e., insufficient/inadequate tumor tissue, tumor content or tumor nucleated cells), failure due to low DNA quality/quantity at extraction, and/or failure after DNA extraction (e.g., failure at DNA library construction, hybridization capture, sequencing/sequencing analyses, etc.; ref. 10). Although failure rates can be improved to an extent through optimizing the tissue testing methodology (15), biopsy procedures are inherently invasive and may not always be feasible in patients with mCRPC because of the low DNA yield obtained from small tumor sample sizes. In addition, the bone-predominant

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Translational Relevance

Our analyses of matched tumor tissue and circulating tumor DNA (ctDNA) samples from patients with metastatic castration-resistant prostate cancer (mCRPC) and *BRCA1*, *BRCA2*, and *ATM* alterations screened in the PROfound study support the consideration of ctDNA testing to identify patients who may be eligible for olaparib treatment. Uniquely, our exploratory analyses of different cutoff values for ctDNA fraction estimates may help guide clinical decisions, supporting clinicians' interpretation of ctDNA test results. This is particularly important when a negative mutation result is returned. This research tool has the potential to help clinicians gain confidence that a negative ctDNA result is not simply due to lack of ctDNA in a provided sample. Although tumor tissue testing remains the gold standard, ctDNA testing could be particularly useful for patients with mCRPC who do not have sufficient or suitable tumor tissue for genomic analyses, or when tissue testing fails to provide a result.

metastatic spread of prostate cancer makes genomic profiling challenging because the process of decalcification can degrade nucleic acids, rendering the sample incompatible for molecular testing (16, 17). Genomic analysis of ctDNA is minimally invasive and is becoming an established molecular technology in precision medicine, with several FDA-approved liquid biopsy assays available for use (18–20).

Our aim was to investigate the feasibility of using ctDNA as an alternative molecular testing solution for profiling mCRPC to identify patients with deleterious alterations in BRCA and *ATM* genes screened in the PROfound study. The results we report will help determine the utility of ctDNA testing as an additional testing method to tissue testing, to guide treatment decisions and help identify patients with prostate cancer who may derive clinical benefit from olaparib treatment, especially those who do not have sufficient or any tumor tissue for molecular testing or whose tumor tissue samples fail molecular screening.

Patients and Methods

The PROfound study design and methodology have previously been published in detail (9, 21). In brief, eligible patients with mCRPC and disease progression on a prior NHA were randomized 2:1 to olaparib tablets [300 mg twice daily (bid)] or a control of physician's choice of enzalutamide (160 mg/d) or abiraterone (1,000 mg/d plus prednisone at 5 mg bid). Patients in Cohort A had at least one alteration on tumor tissue–based testing in *BRCA1*, *BRCA2*, or *ATM*, and patients in Cohort B had alterations in ≥1 of 12 other prespecified genes with a direct or indirect role in HRR: *BARD1*, *BRIP1*, *CDK12*, *CHEK1*, *CHEK2*, *FANCL*, *PALB2*, *PPP2R2A*, *RAD51B*, *RAD51C*, *RAD51D*, and/or *RAD54L*. The primary endpoint of rPFS in Cohort A was assessed by blinded independent central review.

Allocation to Cohort A or B was based on prospective tumor tissue molecular profiling at Foundation Medicine, Inc. (FMI) using the Clinical Laboratory Improvements Amendment (CLIA) HRR clinical trial assay (CTA) or a prior FoundationOne test result (the minor differences between the CLIA HRR CTA and FoundationOne®CDx assays are highlighted in the Supplementary Material). Details of the tumor sample (e.g., organ, collection method, and collection date) were recorded on the specimen collection module in the PROfound study electronic case report form, as completed by participating sites. At screening, matched plasma samples were also collected with patient consent for subsequent analysis, and plasma-derived ctDNA was retrospectively sequenced at FMI using the investigational FoundationOne®Liquid CDx assay. At the time of the study, the CLIA HRR CTA and FoundationOne®Liquid CDx were for investigational use only. Subsequently, the CLIA HRR CTA was bridged to the FoundationOne®CDx test, and both the FoundationOne®CDx and FoundationOne®Liquid CDx assays have now received FDA approval as companion diagnostics (13, 22).

This analysis combines data from the technical assessment pilot study that was performed to determine the feasibility of ctDNA testing using the FoundationOne®Liquid CDx assay and a follow-on clinical validation of the FoundationOne®Liquid CDx for diagnostic development (Supplementary Fig. S1). No changes to the assay were made between the pilot study and the clinical validation stages. The definition of a patient with (i.e., positive for) or without (i.e., negative for) a *BRCA1*, *BRCA2* or *ATM* alteration was based on the presence or absence of the qualifying alteration according to the companion diagnostic label for olaparib (11) for both the tumor tissue and ctDNA assays (23, 24). Patient selection was based on informed consent and sample availability as opposed to demographic or disease characteristics, and all patients who consented and had a sample available were included in this analysis. Sample success was determined by whether a ctDNA next-generation sequencing (NGS) result was obtained.

The PROfound trial was approved by an institutional review board and performed in accordance with the principles of the Declaration of Helsinki, the International Conference on Harmonization Good Clinical Practice guidelines, and the AstraZeneca and Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc. policies on bioethics. All the patients provided written informed consent.

Statistical analysis

Concordance between the FoundationOne®CDx and FoundationOne®Liquid CDx assays was evaluated by calculation of positive percentage agreement (sensitivity), negative percentage agreement (specificity), positive predictive value (PPV), and negative predictive value (NPV). PPV and NPV were calculated using the estimate of *BRCA1*, *BRCA2* or *ATM* alteration prevalence observed in the PROfound study, as well as the sensitivity and specificity calculations (Supplementary Table S1). All statistical analyses were performed using R statistical programming language.

Analysis of ctDNA fraction

The analysis for the quantification of ctDNA fraction (the fraction of circulating DNA in plasma that is tumor derived) has been previously described by Tukachinsky and colleagues (25). In brief, ctDNA fractions were estimated using either of two methods: Tumor fraction estimator (TFE) and maximum somatic allele frequency (MSAF). Two methods were used to increase the sample size where ctDNA fraction estimates could be quantified in this analysis.

TFE methodology (proprietary of FMI; ref. 25) is based on a tumor aneuploidy measure that, for a given sample, incorporates deviations in coverage across the genome. Resulting values are calibrated against a training set based on samples with well-defined tumor fractions to generate an estimate of the tumor fraction. If a lack of tumor aneuploidy limits the TFE's ability to return an informative value, then the MSAF method was used. MSAF calculates the allele fraction of all known somatic, likely somatic, and variants of unknown significance substitution alterations detected at >2,000 median unique coverage by non-PCR duplicate read pairs, excluding germline variants and well-established variants associated with clonal

Table 1. Concordance between tumor tissue and ctDNA testing determined by positive and negative percentage agreements.

	Tissue BRCA/*ATM* mutation detected (T^+)	Tissue BRCA/*ATM* mutation not detected (T^-)	Total
Plasma (ctDNA) BRCA/*ATM* mutation detected (P^+)	143 (81%; T^+/P^+)	24 (8%; T^-/P^+)	167
Plasma (ctDNA) BRCA/*ATM* mutation not detected (P^-)	33 (19%; T^+/P^-)	291 (92%; T^-/P^-)	324
Total	176	315	491
	T^+/P^+: 81% (95% CI, 75–87)	T^-/P^-: 92% (95% CI, 89–95)	PPV = 0.68 NPV = 0.96

Abbreviations: CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

hematopoiesis (CH). Variants with an allele frequency of >90% and 45% to 55% [excluding variants classified as somatic by somatic-germline-zygosity classification (26) based on paired tumor mutation] and the most well established variants associated with CH (27–30) are excluded (for this analysis, all mutations detected in *ASXL1*, *TET2*, and *DNMT3A* were excluded, as well as variants with an allele frequency of <1% for *TP53* to minimize interference of subclonal alterations associated with *TP53*). Variants with the largest allele frequency per patient were used for the remainder of variants reported to calculate MSAF.

ctDNA fraction was considered "evaluable" when a ctDNA fraction score (ranging from 0 to 100) for a patient was obtained by either TFE or MSAF and "not evaluable" if it was not obtained by either TFE or MSAF. ctDNA fraction results were used to assess sensitivity and specificity at patient level, at different ctDNA fraction cutoff points (i.e., not evaluable, <10% and ≥10%), and at variant-subtype level to determine if ctDNA fraction differs according to variant type reported for BRCA and *ATM* genes.

Gene-specific zygosity

Gene-specific zygosity was determined using an exploratory computational algorithm developed at FMI from the FoundationOne®CDx tissue test and is previously reported (26). Patients were classified in one of the following subgroups based on the evidence for a second hit in the same BRCA gene: biallelic, heterozygous, or unknown. A detailed explanation of criteria for patient classification is available in Supplementary Table S2. In brief, the biallelic subgroup includes patients with homozygous deletions, homozygous mutations, a pathogenic mutation with no evidence of a wild-type allele, or those with two alterations but no evidence of whether they occur in the same or different alleles (suspected biallelic inactivation). All patients in the heterozygous subgroups are considered suspected heterozygous because, although the presence of a wild-type allele was determined by the genomic method used, it is not possible to rule out that the other allele may have been inactivated by alterations not detectable by the targeted NGS assay.

Data availability

The data generated in this study are not publicly available because of patient privacy but are available upon reasonable request in accordance with AstraZeneca's data sharing policy described at https://astrazenecagrouptrials.pharmacm.com/ST/Submission/Disclosure.

Results

Patient population and generation of ctDNA assay results

The subset population of patients screened in PROfound in which ctDNA testing was performed consisted of 619 patients in total: 229 patients with and 390 without deleterious alterations in *BRCA1*, *BRCA2* or *ATM* genes reported in tissue (Supplementary Fig. S1). Of samples tested, and from which a matched tumor tissue result was available, 503 were primary tumor samples, 93 indicated soft tissue metastasis, 19 indicated bone metastasis, and 4 patients were of an unknown stage of disease.

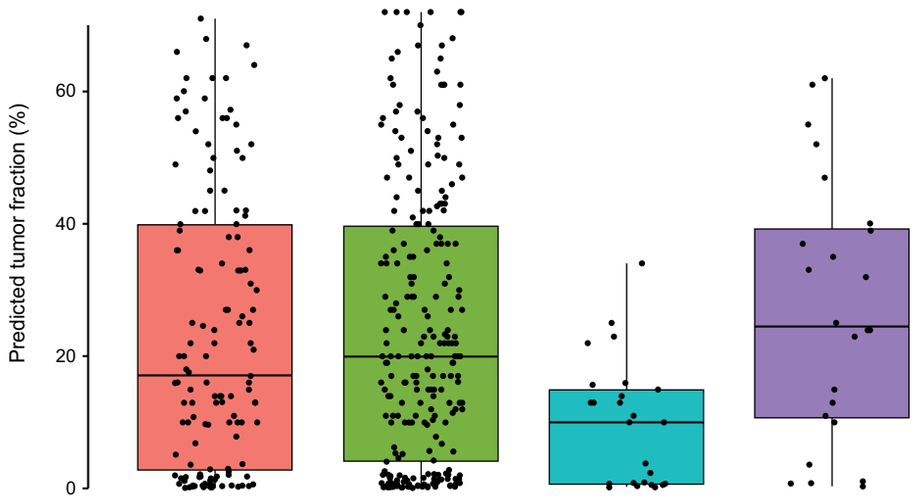
In total, 81% (503/619) of ctDNA samples tested by the FoundationOne®Liquid CDx assay yielded an NGS result. The proportion of samples that yielded results for the assay was higher (82% vs. 69%) when a volume of plasma (regardless of ctDNA fraction values) of ≥7 mL was available for testing, a volume more representative of the input criteria for FoundationOne®Liquid CDx required in the diagnostic setting (Supplementary Fig. S2). Notably, a higher rate of 90% (503/558) was achieved when the impact of a technical failure in the clinical sample-processing procedure, which affected 61 samples, was excluded (see Discussion and Supplementary Material for further information).

Tissue and ctDNA concordance

Using the tumor tissue result as reference, the sensitivity, based on the presence of BRCA or *ATM* alterations in tumor tissue, compared with ctDNA, was 81% (143/176), and the specificity, based on the absence of BRCA or *ATM* alterations in tumor tissue, was 92% (291/315). Nineteen percent (33/176) of patients had BRCA or *ATM* alterations detected in tumor tissue that were not detected in ctDNA (T^+/P^-), and 8% (24/291) of patients had BRCA or *ATM* alterations detected in ctDNA that were not detected in tumor tissue (T^-/P^+; **Table 1**). The PPV was 0.68 (i.e., 32% of ctDNA BRCA- or *ATM*-positive patients are predicted to be negative by tumor tissue testing), and the NPV was 0.96 (i.e., 4% of ctDNA BRCA- or *ATM*-negative patients are predicted to be positive by tumor tissue testing; **Table 1**). Sensitivity and specificity rates for the pilot study compared with the clinical validation study were relatively consistent and are described in Supplementary Table S3. Sensitivity and specificity were also assessed based on clinical characteristics: metastatic tumors and primary tumors, as well as archival tumor tissue and newly collected tumor tissue (Supplementary Tables S4 and S5, respectively); however, interpretation was challenging because of the imbalance in sample size of the groups. Assay sensitivity also remained high regardless of gene specific zygosity classification of BRCA or *ATM*-positive patients by tumor tissue testing (Supplementary Table S6).

Evaluation of ctDNA fraction

Of the 491 samples that had a biomarker result for both tumor tissue and ctDNA, 428 (87%) had an evaluable ctDNA fraction estimate. For 63/491 (13%) samples, a ctDNA fraction estimate could not be generated (i.e., not evaluable) by TFE or MSAF. Where a ctDNA fraction estimate was evaluable, the median ctDNA fraction estimate was 18% [interquartile range (IQR) 2.8%–38%; **Fig. 1**]. A lower proportion of T^+/P^- cases (76%; 25/33) had an evaluable ctDNA fraction estimate relative to T^-/P^+ cases (100%; 24/24) and T^+/P^+ cases (93%; 133/143; **Fig. 1**). Where estimated ctDNA fractions were



	T^+/P^+	T^-/P^-	T^+/P^-	T^-/P^+	Total
ctDNA tumor fraction evaluable, *n*/*N* (%)	133/144 (93%)	246/291 (84.5%)	25/33 (75.8%)	24/24 (100%)	**428/491 (87%)**
Median ctDNA fraction estimate where tumor fraction was evaluable, % (IQR)	17% (2.9–40)	20% (4.1–39.8)	10% (0.8–15)	24.5% (10.8–39.3)	**18% (2.8–38)**

Figure 1.
Evaluable ctDNA fraction rate and predicted tumor fraction (%) in all cases in which a biomarker result was obtained for both tissue and ctDNA: concordant (T^+/P^+, T^-/P^-) and discordant (T^+/P^-, T^-/P^+) cases.

evaluable, median ctDNA fraction estimates were lower in T^+/P^- cases (10%) and higher in T^-/P^+ cases (24.5%). Median ctDNA tumor fraction estimates for samples included in sensitivity analysis (17%) and specificity analysis (20%) were relatively consistent (**Fig. 1**).

Using the tumor tissue result as reference, sensitivity and specificity were assessed at four different ctDNA fraction estimate thresholds. At estimated ctDNA fraction thresholds of ≥10% and ≥1% to <10%, sensitivity values were 87% and 92%, respectively, and the corresponding specificity values were 90% and 95%, respectively. When ctDNA fraction was <1% and not evaluable, the sensitivity values were 68% and 56%, respectively, and the corresponding specificity values were 92% and 100%, respectively (Supplementary Table S7).

Gene and variant subtype detection comparison in tumor tissue and ctDNA

Using the tumor tissue result as reference, the gene-specific level of concordance between tumor tissue and ctDNA was similar for *BRCA2* and *ATM* genes, with the highest rate of sensitivity (82%) observed in *BRCA2* and the highest rate of specificity in *BRCA1* (99%) and *BRCA2* (98%) relative to *ATM* (95%; Supplementary Table S8). This concordance analysis was expanded to assess HRR alterations reported in tumor tissue and ctDNA in 12 other genes. The level of specificity was high (i.e., >95%) for all genes (Supplementary Table S8; Supplementary Fig. S3). This exploratory analysis was limited by sample size to assess assay sensitivity in HRR genes beyond BRCA and *ATM* due to the nature of this study design, that is, patients were selected on the basis of presence or absence of deleterious alterations in BRCA or *ATM* genes reported in tumor tissue. Therefore, this does not represent natural prevalence of these gene mutations in mCRPC. For this reason, analysis in the study focuses predominantly on BRCA and *ATM* genes. The OncoPrint (31) in **Fig. 2** shows patient-matched tumor and ctDNA sample results specific to BRCA and *ATM* alteration detection with overlayed ctDNA fraction estimates per patient.

Of the 491 patients who had a biomarker result for both tumor tissue and ctDNA, 200 patients had an alteration reported in BRCA or *ATM* genes. Of these, 197 variants were reported in tumor tissues of 176 patients and 207 variants were reported in the ctDNA of 167 patients, respectively. The relative proportion of different variant subtypes reported in ctDNA and tissue was comparable to that of PROfound Cohort A, with the exception of homozygous deletions, which was low for ctDNA (3%) relative to tumor tissue (14%–15%; **Fig. 3**).

Using tissue as reference, at the variant level, a high rate of overall sensitivity between tissue and ctDNA was observed across BRCA and *ATM* alterations (74%). Particularly high sensitivity was observed for frameshift/indels (86%), nonsense (93%) and splice (87%) alterations; data were limited for missense alterations (**Table 2**). The rate of sensitivity observed for other alterations was lower, particularly for large structural alterations, that is, homozygous loss (27%) and large rearrangements (63%; **Table 2**, Supplementary Table S9; **Fig. 2**). The equivalent values when ctDNA was used as reference are shown in **Table 2** and Supplementary Table S10.

Although the overall rate of concordance was high (71%; 146/207; **Table 2**), it was lower for alterations detected in ctDNA with a low variant allele frequency (VAF). Discordance was enriched for individual variants detected in ctDNA only in *ATM* below a VAF of 1% (**Fig. 4**). These variants would not reach the lower limit of detection if

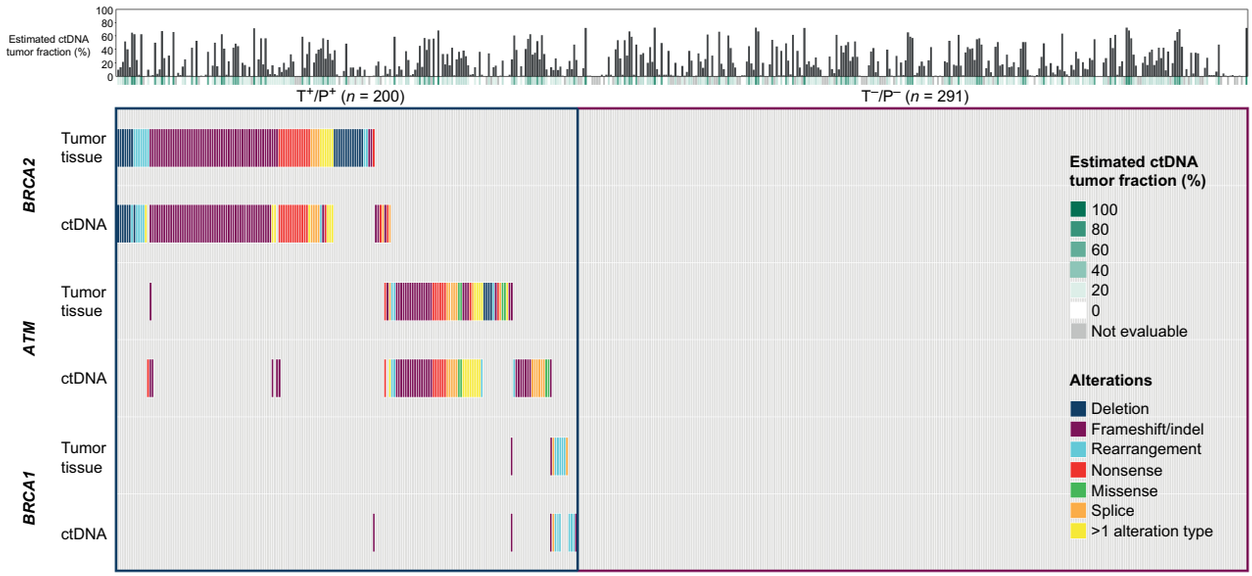


Figure 2.

High rate of concordance demonstrated between tissue and ctDNA in both the absence and presence of *BRCA1*, *BRCA2*, and *ATM* genes across 491 patient-matched samples. The OncoPrint (31) shown illustrates the tissue or plasma-positive population ($n = 200$) on the left (patient samples are ordered by T^+/P^+, T^+/P^-, and T^-/P^+, respectively, per gene) and the tissue- and plasma-negative population on the right ($n = 291$), demonstrating the high level of concordance observed in both the presence and absence of BRCA and *ATM* alterations. Estimated ctDNA fraction is indicated by the bar chart and heatmap above the OncoPrint. In the heatmap, gray bars are cases where estimated ctDNA fraction is not evaluable. Variant subtypes detected for each gene are color coded. Yellow bars are cases where more than one genetic alteration is detected in either tumor tissue or ctDNA for a given gene, when applicable.

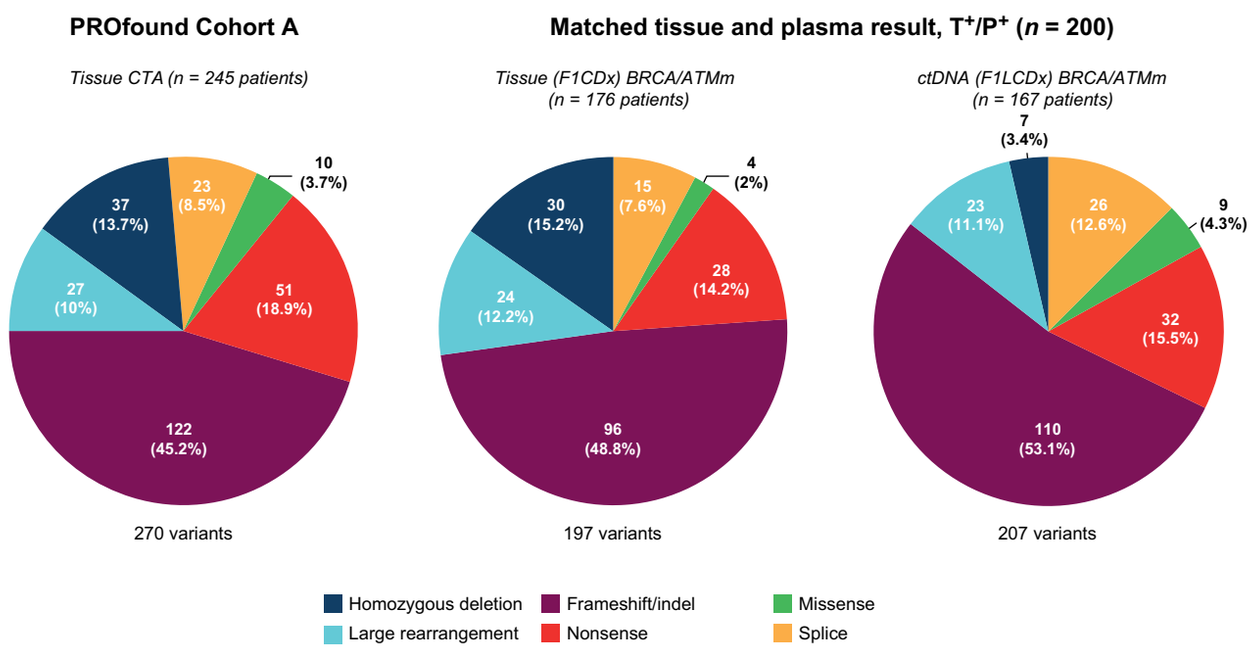


Figure 3.

Proportion of variant subtypes detected in tissue for which a biomarker result was also available in plasma for BRCA/*ATM* genes was comparable to that of PROfound Cohort A, except for homozygous deletions. The splice-site variants in PROfound Cohort A include one synonymous alteration and one intronic alteration. F1CDx, FoundationOne®CDx; F1LCDx, FoundationOne®Liquid CDx; m, mutation.

Table 2. BRCA/*ATM* variant subtype detection sensitivity in ctDNA and tissue.

	Sensitivity of detection in tumor tissue			Sensitivity of detection in ctDNA		
Variant types	**Detected in tumor tissue**	**Detected in tumor tissue and ctDNA**	**Detected in tumor tissue only**	**Detected in ctDNA**	**Detected in tumor tissue and ctDNA**	**Detected in ctDNA only**
Frameshift/indel	96	83 (86%)	13 (14%)	110	83 (75%)	27 (25%)
Homozygous loss	30	8 (27%)	22 (73%)	7	7 (100%)	0
Large rearrangement	24	15 (63%)	9 (37%)	23	16 (70%)	7 (30%)
Nonsense	28	26 (93%)	2 (7%)	32	26 (81%)	6 (19%)
Splice	15	13 (87%)	2 (13%)	26	13 (50%)	13 (50%)
Missense	4	1 (25%)	3 (75%)	9	1 (11%)	8 (89%)
Total	197	146 (74%)	51 (26%)	207	146 (71%)	61 (29%)

the same variant was detected at this VAF in tumor tissue (i.e., <1%). These mutations were often co-occurring events in tumor, that is, the tumor contained an additional alteration that was detected in both tumor tissue and ctDNA, representing possible second hits or subclonal variants (**Figs. 2** and **4**). An enrichment of variant detection in tissue and ctDNA was also observed at 50% VAF, which is consistent with potential germline mutations (**Fig. 4**).

Where estimated ctDNA tumor fraction was evaluable, ctDNA fraction estimates were lower for variants detected in tissue only (median 2.3%; IQR 0.6%–13.3%) relative to variants detected in both tumor tissue and ctDNA (median 18%; IQR 5%–40%) and variants detected in ctDNA only (median 23%; IQR 10%–40%; Supplementary Fig. S4). This pattern was particularly evident for large structural alterations, that is, homozygous deletions and large rearrangements (**Fig. 5**), limiting the reporting sensitivity of *BRCA2* homozygous deletions with low estimated ctDNA fraction rates.

Discussion

We report here genomic profiling analysis investigating sensitivity and specificity between matched tumor tissue and ctDNA testing using samples from patients with mCRPC screened for the PROfound study. This analysis is important because of the large number of tissue samples that fail molecular screening (31% of tumor tissue samples failed in the PROfound study).

Initially, we analyzed the ability of the FoundationOne® Liquid CDx assay to generate an NGS result and found that the assay yielded biomarker results for a high proportion of samples (81%). An unexpected technical failure in a laboratory sampling process prevented a potentially higher proportion of samples from yielding a result (90% of samples yielded a test result when the 61 samples lost in a single batch as a result of process failure of a single assay plate were removed from the analysis). Nevertheless, despite the failure, the effectiveness of the assay in identifying additional potential patients with mCRPC who could benefit from ctDNA-based testing was demonstrated.

Second, we evaluated concordance between tumor tissue testing compared with ctDNA testing in matched samples, in both the presence and absence of *BRCA1*, *BRCA2*, and *ATM* alterations, and found that a high rate of sensitivity (81%) and specificity (92%) was evident, using the tumor tissue result as reference.

To aid our evaluation of this ctDNA technology, exploratory tumor fraction analysis was integrated into our study. ctDNA fraction estimates were not evaluable for 13% of samples in which an NGS biomarker result was reported. These patients are likely ctDNA nonshedders with very low tumor burden (32). ctDNA fraction data were also integrated into our concordance analysis. We found that the non-evaluable (likely non-shedding) cases were enriched in our discordant patient population where BRCA or *ATM* alterations were detected in tumor but not in ctDNA (T^+/P^-). When ctDNA fraction was evaluable for this patient population (T^+/P^-), tumor fraction levels were much lower than in the rest of the patient population. When assessing concordance at four different estimated ctDNA fraction thresholds (i.e., not evaluable, <1%, ≥1% to <10%, and ≥10%), we demonstrated that as higher ctDNA fraction estimates were imposed, assay sensitivity increased considerably whereas assay specificity was stable and remained high.

When assessing individual genes, a high rate of sensitivity and specificity was also observed, although sample numbers for *BRCA1* were small as these alterations occur less frequently in mCRPC than *BRCA2* and *ATM* alterations (33). In cases where discordance was observed, the underlying explanations differed depending on presence of BRCA and *ATM* alterations in tumor tissue and ctDNA. Where patients were observed who were positive for BRCA or *ATM* in tumor tissue but negative in ctDNA (T^+/P^-), affecting assay sensitivity, it was predominantly attributed to limitations in structural variant detection (e.g., homozygous deletions and large rearrangements) in ctDNA, which are more common events in *BRCA2*-mutant tumors.

We also identified likely low or non-shedding ctDNA to be a root cause for T^+/P^- cases (as discussed above). This pattern of low predicted tumor fraction was striking for patients with homozygous deletions and large rearrangements detected in tumor tissue only. Our study shows that detection of structural variation is possible using the ctDNA we evaluated, but there is a need for improvement in this area. Identification of homozygous deletions using FoundationOne® Liquid CDx is currently validated for *BRCA1*, *BRCA2*, and *PTEN* genes but not for other genes in the panel. Furthermore, the importance in detecting structural variants was emphasized in the TOPARP-B clinical trial, whereby the patients who received the greatest benefit from PARP inhibitor (olaparib) treatment had homozygous deletions in *BRCA2* (34).

Conversely, where discordance was observed between BRCA or *ATM* alterations identified by ctDNA but not seen in tumor tissue, affecting test specificity, this was predominantly attributed to low VAF observed for variants reported in ctDNA. These variants would fall below the lower limit of detection if reported at the same frequency as for the tumor tissue assay (<1%). These alterations were often co-occurring events in patients in whom an additional alteration was detected in ctDNA, representing possible second hits or subclonal passenger variants (**Fig. 5**). This pattern was notably enriched for patients harboring *ATM* alterations; however, the relevance of these observations is not currently known.

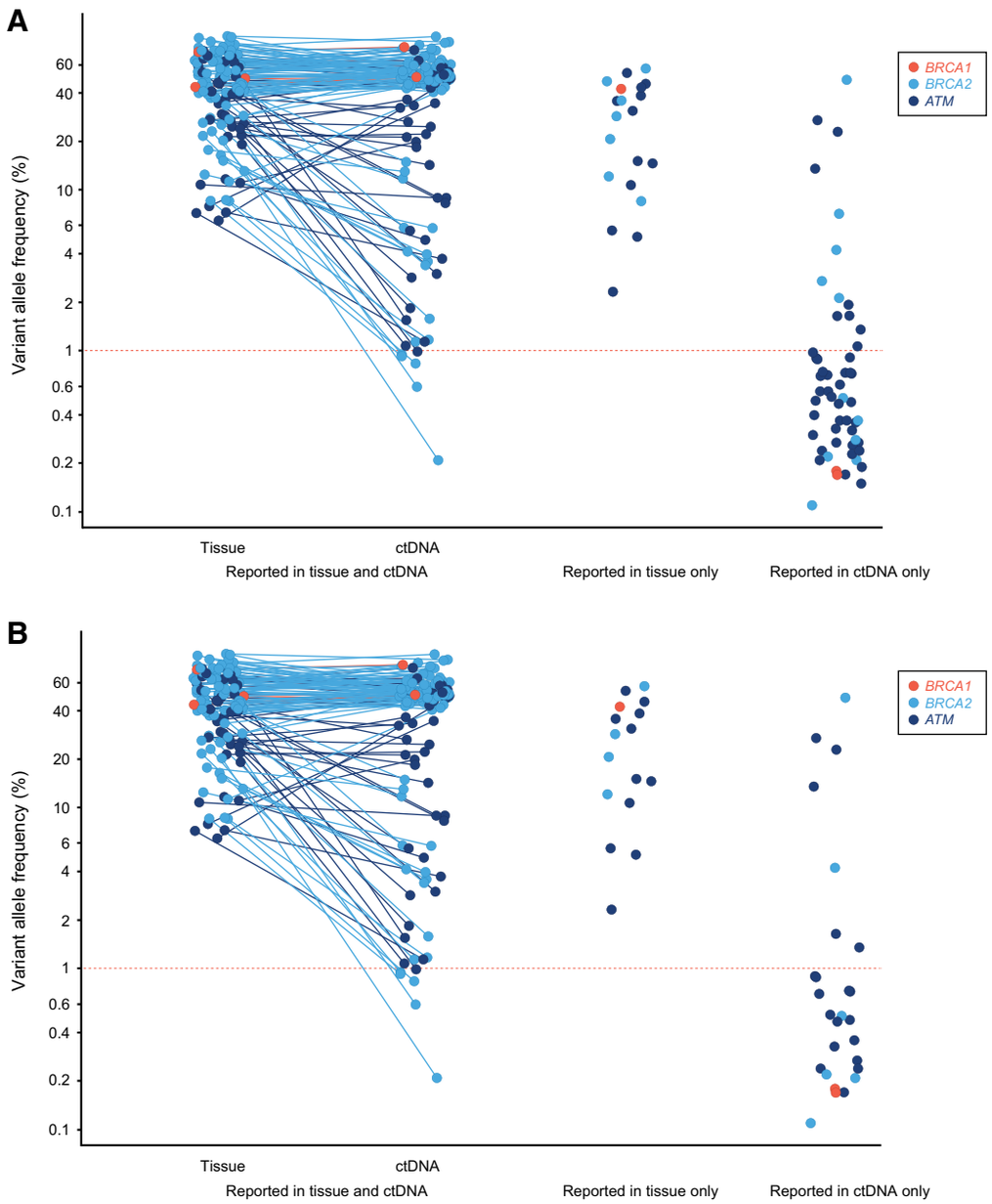


Figure 4.

A, Variants detected in tissue and plasma in BRCA/*ATM* and (**B**) variants detected in tissue and plasma in BRCA/*ATM* discordant cases where an additional variant for that patient is detected in tissue and removed. **A** and **B,** Each dot represents a variant reported in BRCA and *ATM* genes that is color coded. Concordance between tumor tissue and ctDNA is represented by adjoining lines between dots in the first and second columns. Variants reported in tumor tissue only and ctDNA only are represented by individual dots in the third and fourth columns, respectively. The VAF reported for each variant is represented on the *y*-axis in logarithmic scale, and the red horizontal line represents 1% VAF. Patient-level concordance for variants reported is considered in **B**. Discordant variants whereby co-occurring variants are detected in both tumor tissue and ctDNA (i.e., concordant at patient level) have been removed. These cases were more common for *ATM* (dark blue) alterations relative to BRCA (light blue) variants and were enriched at low VAF.

ctDNA testing technology continues to be developed and challenges remain. For example, further optimization of the assay is necessary as a proportion of patients (∼20% in our study) with qualifying mutations for olaparib treatment were identified through tissue testing producing a negative result via ctDNA assay. In the clinical environment, these patients with false negative ctDNA results could be overlooked for personalized treatment they may derive benefit from. If ctDNA testing is negative, such patients could be considered for additional tissue testing in the form of a repeat biopsy or reflexively testing primary archival tissue if available and not already attempted (35). In addition, variants of CH interference are another challenge. Although research has suggested that some variants in *ATM* are associated with CH (36), it is not possible to definitively classify these variants as such without a germline result using DNA from the buffy coat as sample input, the

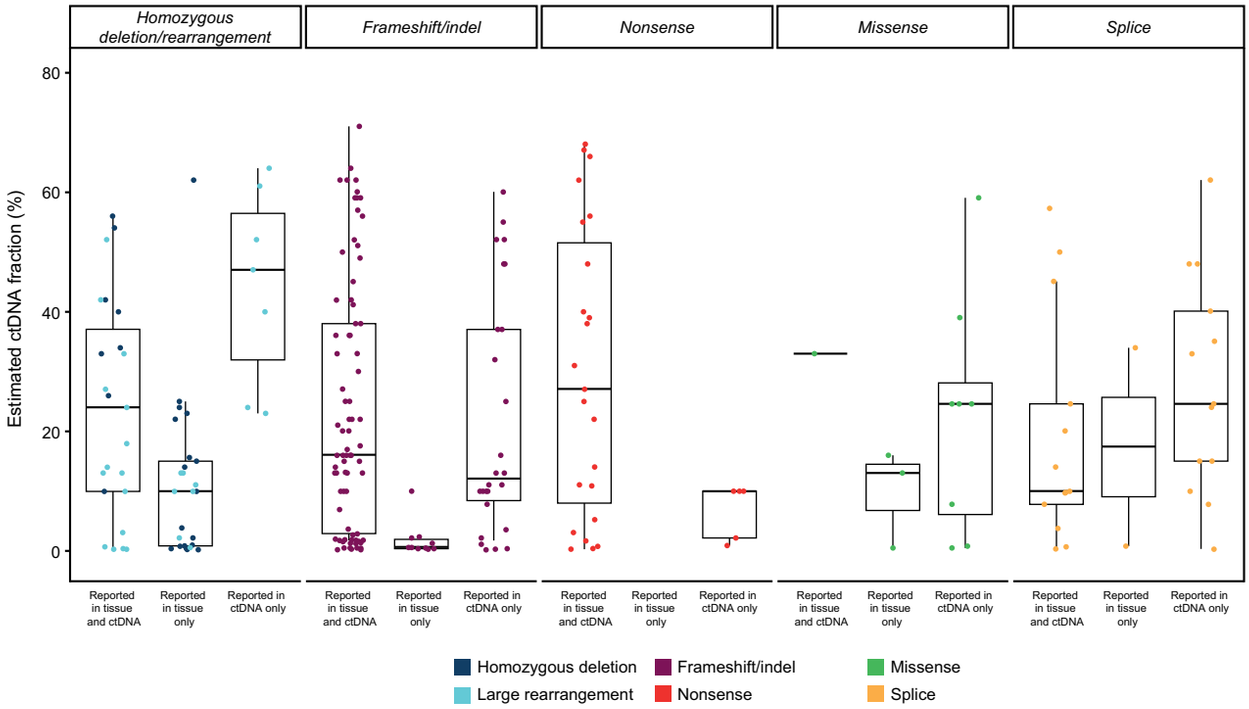


Figure 5.
Estimated ctDNA fraction in variant subtypes detected in tissue and ctDNA (left), variants detected in tissue only (middle), and variants detected in ctDNA only (right) for individual variant subtypes. The figures highlight that the ctDNA fraction estimates are particularly low for large structural alterations detected in tissue only.

lack of which is a limitation of the PROfound study design. Although caution is advised in interpreting *ATM* alterations at this low frequency, definitive classification of these variants as CH can only be speculative at this time. In addition, the clinical relevance of CH interference in prostate cancer is an area that still needs to be further elucidated.

The data on ctDNA fraction estimates suggest that when low ctDNA fraction estimates are reported, it can affect the detection rates of certain genetic alterations in ctDNA. Although this analysis is exploratory, this finding has the potential to help interpretation of a negative ctDNA test result in the future. If the ctDNA fraction estimate is too low, then a patient with potential genetic alterations who would potentially benefit from treatment with a PARP inhibitor may be overlooked. This also highlights that although the use of ctDNA testing in patients with mCRPC offers an additional testing solution to tumor tissue testing, it is not a replacement for it. Tissue testing remains the gold standard, with ctDNA testing a particularly useful method for patients who have insufficient or no tumor tissue available for testing, or when tissue testing fails to provide a result. Previous research has demonstrated that the efficacy of olaparib in patients enrolled in PROfound with *BRCA1*, *BRCA2*, and *ATM* alterations identified by ctDNA testing was similar to that for the Cohort A population in PROfound, whose alterations were identified by tissue testing (37). Tissue and ctDNA testing involve different methodologies and therefore have their own inherent limitations (as described in Vandekerkhove and colleagues; ref. 38), and as neither test captures all clinically relevant alterations in all patients, it is to the clinicians' advantage that both testing methods are available.

It is important to highlight, however, that quantifying ctDNA tumor fraction, especially in the context of low ctDNA tumor fraction percentages, is currently not always possible with this assay. As stated, in the PROfound patient population, which is a metastatic setting, estimating the ctDNA fraction was not possible for 13% of samples. Genomic sequencing techniques such as low-pass whole-genome sequencing may be able to identify genomic alterations (including homozygous deletions) in samples of low tumor fraction percentages (39); however, the evidence highlighting the clinical utility of such techniques in precision oncology is limited (40). Although there is considerable evidence related to the prognostic potential of ctDNA tumor fraction assessment in mCRPC (32, 41, 42), these data, as well as other research (43), highlight that additional work is needed to optimize and standardize quantification, particularly when tumor fraction/shedding rates are particularly low.

Comparison with other studies

In PROfound, 31% of patients' tumor tissue samples failed molecular screening (9, 21). This is not unusual as biopsy failure rates between 25% and 75% have been reported for other studies involving genomic selection of patients, which potentially limits treatment decision making, especially in patients with mCRPC and non-accessible lesions (44–46). Although ctDNA screening methodology is still in its early stages, analysis of ctDNA has been used to characterize and monitor disease, as well as predict outcome and treatment response in several solid malignancies. For example, in mCRPC, the ctDNA fraction at baseline and associated changes during treatment have been strongly linked to a shorter time to progression and OS (41). Numerous studies have also shown high

concordance of molecular alterations and copy number changes in the ctDNA of patients with mCRPC compared with somatic alterations previously identified through profiling of metastatic tissue (3, 47–49). In addition, a review of blood-based liquid biopsies to inform clinical decision making in prostate cancer concluded that liquid biopsies have clinical utility as a source of prognostic, predictive and response biomarkers, although standardization of assays and analytical/clinical validation is necessary before clinical implementation (50). These studies, and our investigations reported here, suggest that ctDNA testing has potential as an additional tool in identifying *BRCA1*-, *BRCA2*- and/or *ATM*-altered mCRPC to guide treatment decisions.

The median ctDNA fraction estimate we observed for this population (18%; IQR 2.8%–38%) was very similar to that reported in TRITON2 (18.1%; IQR 1.5%–38.1%), which investigated the PARP inhibitor rucaparib in patients with mCRPC and a *BRCA1* or *BRCA2* alteration (25). The equivalent result in TRITON3 was 3.4%; the difference could be reflective of a greater amount of ctDNA shedding, representing patients with higher tumor burden after more lines of therapy (51) as TRITON3 patients had disease progression on one prior hormone therapy, whereas TRITON2 patients had disease progression on one to two lines of hormone therapy followed by a taxane-based chemotherapy in the castration-resistant setting (25). It has also been highlighted by Annala and colleagues (32) that low/unquantifiable TF levels themselves have also been associated with improved patient prognosis in prospective clinical trials. However, it is important to note that ctDNA fraction estimation is still limited in exact quantification; therefore, comparison between trial datasets should be done with caution.

Study limitations

This study was designed to assess concordance in both the presence and absence of BRCA and *ATM* alterations using the tumor tissue result as a reference. The prevalence of BRCA and *ATM* alterations in patients with mCRPC is approximately 15% (3, 8, 52), whereas the BRCA and *ATM* population in this study represents approximately 36% of patients where matched data are available. Therefore, the study cohort is not representative of natural BRCA or *ATM* prevalence, and the data should not be interpreted as such. This also limits our ability to assess concordance effectively using the ctDNA result instead of tissue as reference, hence why PPV and NPV algorithms based on BRCA and *ATM* prevalence in PROfound are used to facilitate this. This study design also limits our ability to assess assay sensitivity (using tissue as reference) in other genes beyond BRCA or *ATM* that are directly or indirectly involved in HRR, as has been discussed in the results section. Another limitation of our study is that whether the finding of low minor allele frequency events by ctDNA testing are true tumor events cannot be discerned; a very low ctDNA fraction and detection of low-level *ATM* alteration may also signify CH. Also, some of the tumor tissue samples were archival and were therefore not taken at the same time as the ctDNA sample. Although the scientific literature predominantly states that genomic alterations associated with HRR (namely, *BRCA1* and *BRCA2* genes) are truncal mutational events (53), some studies have recently indicated that certain *BRCA2* alterations emerge after abiraterone therapy and are therefore not detectable in diagnostic samples (54). In addition, our analysis only evaluated tissue samples that passed a quality assurance requirement for tissue-based testing (1,255 patient samples that failed tumor testing were excluded); therefore, we could not fully assess the utility of ctDNA-based sequencing in this context.

Conclusions

The overall ability of the ctDNA assay to generate an NGS result in plasma samples from patients in the PROfound study was high. This suggests that ctDNA testing has the potential to greatly complement tumor tissue NGS testing in the identification of patients with mCRPC and *BRCA1*, *BRCA2*, and *ATM* alterations who are potentially suitable for receiving targeted PARP inhibitor treatments that may improve outcomes compared with standard treatments. ctDNA testing may be particularly useful for patients with insufficient or no tumor tissue for genomic analysis, when tissue testing fails to provide a result, or when the tissue sample characteristics are not ideal (e.g., an aged archival sample or of bone instead of soft tissue). The ability of the ctDNA test to detect structural variation alterations, including BRCA homozygous deletions, notably at low ctDNA fraction percentages, was limited and remains a technical challenge for further improvement.

Tumor tissue testing continues to be the gold standard for molecular testing, and our results support consideration of the ctDNA assay as an additional tool in identifying *BRCA1*-, *BRCA2*- and *ATM*-altered genes in patients with mCRPC to guide treatment decisions.

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Note

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