Cell-free DNA (cfDNA) variant analysis is used to guide treatment decisions for men with metastatic prostate cancer (mPC) and to enroll patients on clinical trials. Two poly(ADP) ribose polymerase inhibitors (PARPi) were recently granted US Food and Drug Administration (FDA) approval for use in selected patients with mPC based on DNA repair gene status: rucaparib for patients with \textit{BRCA1} or \textit{BRCA2} variants and olaparib for patients with \textit{ATM}, \textit{BRCA1}, \textit{BRCA2}, \textit{BARD1}, \textit{BRIP1}, \textit{CDK12}, \textit{CHEK1}, \textit{CHEK2}, \textit{FANCL}, \textit{PALB2}, \textit{RAD51B}, \textit{RAD51C}, \textit{RAD51D} or \textit{RAD51L} variants. Following these biomarker-guided approvals we expect cfDNA testing will sharply increase for patients with mPC because it offers the convenience and simplicity of testing on a blood sample in the advanced disease setting.\textsuperscript{1,3,4} Thus, there is an urgent need to understand cfDNA testing performance and sources of test interferences.

Clonal hematopoiesis of indeterminate potential (CHIP) is a known confounder of cfDNA testing.\textsuperscript{5,6} Clonal hematopoiesis of indeterminate potential variants are detected in both plasma and whole blood, whereas prostate cancer variants are detected in plasma only. Yet most commercial labs perform cfDNA testing using a plasma-only approach that cannot reliably distinguish variants derived from prostate cancer vs those arising from CHIP. To improve cfDNA assay performance, we developed an approach (UW-OncoPlexCT) that simultaneously analyzes plasma and paired whole-blood control samples.\textsuperscript{4} Using this paired testing approach we sought to de-
termine to what degree CHIP interferes with the results of prostate cancer cfDNA testing.

## Methods

We retrospectively reviewed cfDNA study results from 69 patients with advanced prostate cancer (metastatic disease or with rising PSA following localized therapy) sequenced by our Clinical Laboratory Improvement Amendments (CLIA)-certified and College of American Pathologists (CAP)-accredited clinical UW-OncoPlexCT protocol. Plasma cfDNA and a paired whole-blood control sample were tested in every patient. We defined CHIP interference as a pathogenic variant with variant allele fractions (VAFs) of at least 2% in both the whole blood and plasma. Germline variants were distinguished from CHIP clones by tumor sequencing. Sequencing data analysis and variant interpretation were performed by an expert molecular pathologist (C.C.P.). All data were manually reviewed in the integrated genomics viewer (IGV) to exclude sequencing artifacts. Data were generated and preprocessed by the University of Washington NGS Laboratory and Analytics group. This study was performed in accordance with the Declaration of Helsinki guidelines and approved by the University of Washington Fred Hutchinson Cancer Consortium institutional review board and all patients provided written informed consent.

## Results

We detected CHIP interference clones at least 2% variant fraction in 13 of 69 patients (19%; 95% CI, 10%-30%). Seven patients (10%; 95% CI, 4%-20%) had CHIP variants in DNA repair genes that are used for PARPi selection (ATM n = 5, BRCA2, n = 1 and CHEK2, n = 1) (Figure) (Table). The 6 remaining patients had CHIP interference in genes frequently impacted by CHIP: ASXL1, DNMT3A, PTEN, TET2, and TP53 (Figure) (eFigure in the Supplement).

We observed that CHIP interference correlated exponentially with increasing age (R² = 0.82). We detected CHIP in 0% (0/6) of men aged 40 to 50 years, 12.5% (2/16) of men aged 51 to 60 years, 6.3% (1/16) of men aged 61 to 70 years, 20.8% (5/24) of men aged 71 to 80 years, and 71% (5/7) of men aged 81 to 90 years (Figure, A). In 20 patients with advanced prostate cancer, we detected a total of 23 pathogenic variants in DNA repair gene variants used for selection of PARPi therapy, from the following source(s): CHIP interference somatic (n = 8, 1 patient had 2), non-CHIP somatic (n = 9), germline (n = 6) (Figure, B). We considered germline variants and non-CHIP somatic variants as true positives (n = 15) and CHIP interference as false positives (n = 8). Restricting the assay to a plasma-only analysis, only 65% of DNA repair gene variants detected were true positives (15/23). When incorporating a paired whole-blood control to remove CHIP interference, all DNA repair gene variants were true positives (15/15, 100%).

The patient with BRCA2 CHIP interference had cfDNA testing done in parallel by an outside commercial laboratory using a plasma-only assay, which was unknown to our laboratory at the time of testing. The BRCA2 CHIP clone was clinically reported by the commercial lab with the recommendation to use PARPi therapy.

## Discussion

We found that a strikingly high proportion of DNA repair gene variants in the plasma of patients with advanced prostate cancer are attributable to CHIP. The CHIP variants were strongly correlated with increased age, and even higher than expected by age group. The high rate of CHIP may also be influenced by prior exposure to chemotherapy. We are concerned that CHIP interference is causing false-positive cfDNA biomarker assessments that may result in patient harm from inappropriate treatment, and delays in delivering alternative effective treatment options. Without performing a whole-blood control, 7 of 69 patients (10%) would have been misdiagnosed and incorrectly deemed eligible for PARPi-inhibitor therapy based on CHIP interference in plasma. In fact, 1 patient in this series had a BRCA2 CHIP clone that had been previously reported by a commercial lab with the recommendation to use a PARPi. To mitigate these risks, cfDNA results should be compared to results from whole-blood control or tumor tissue.

Challenges of accurate cfDNA testing are beginning to be described. A recent report highlighted inaccuracies of commercial laboratory cfDNA testing in patients with prostate cancer. In that report, cfDNA samples from 40 patients were sent to 2 separate CLIA-certified laboratories and only 9 of 40 (23%) demonstrated congruence (complete or partial) of positive findings. The consistent findings included ATM and TP53 variants in patients with low PSA at the time of blood draw, raising suspicion that these may be CHIP clones. The CHIP interference in cfDNA testing has also been reported in other cancer types. In renal-cell carcinoma (RCC), for example, CHIP was found to affect cfDNA results in 43% of patients.

Overall, ATM accounted for the majority of clinically relevant CHIP interference in our series. The ATM gene has...
been described as a frequent CHIP clone in clinical cancer predisposition testing, along with CHEK2 and TP53. We speculate that CHIP interference in cfDNA testing could be affecting results of PARPi clinical studies of patients with metastatic prostate cancer. Trials allowing plasma-only cfDNA testing for enrollment may have included patients with false-positive results associated with CHIP in DNA repair genes, particularly in ATM. We speculate that this could be contributing to low PARPi response rates reported in patients with ATM variants, such as recently reported from the TRITON2 study.

**Limitations**

This study has several limitations including relatively small sample size, the retrospective nature of the study, and heterogeneity in patient populations and prior therapies.
Conclusions

Findings of this study suggest that CHIP substantially interferes with plasma cfDNA testing in patients with advanced prostate cancer. There is a risk for widespread misdiagnosis and overtreatment of men with PARPi using currently available commercial cfDNA assays. We recommend that all cfDNA testing in patients with prostate cancer include a whole-blood control to distinguish CHIP from prostate cancer variants.

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