## Supplementary methods

Blood processing and DNA isolation

Blood samples collected in Streck tubes were centrifuged at 300g for 20 minutes at room temperature and plasma was transferred to a new conical tube. Next, the plasma was centrifuged at 5000g for 10 minutes and transferred to 2 conical tubes, labeled and stored at -80℃. Total cell-free DNA (cfDNA) was extracted from plasma using the QIAamp Circulating Nucleic Acid Kit (Qiagen) according to the manufacturer's protocol and eluted in 45µL low-TE buffer. The DNA concentration was quantified using a HS dsDNA Qubit assay (Thermofisher) and samples with a high cfDNA concentration were checked for genomic contamination on a Fragment Analyzer (Agilent high sensitivity genomic DNA kit #DNF-488-0500). White blood cell (WBC) DNA was extracted from whole blood EDTA tubes using an automated nucleic acid isolation on a Chemagen-Hamilton robotic system. WBC DNA was eluted in chemagen Elution Buffer (CMG-1756) and diluted using low-TE buffer. The WBC DNA concentration was quantified using a BR dsDNA Qubit assay (Thermofisher).

Library preparation and sequencing

Sequencing libraries were prepared from 10-100 ng of DNA input per sample, depending on the overall yield from DNA extraction (**Supplementary Table S2**). WBC DNA libraries (all 100 ng) were prepared with the KAPA Hyper Plus Kit and underwent enzymatic fragmentation (15 minutes at 37℃) to ~180 bp. Plasma cfDNA libraries were prepared with the KAPA Hyper Prep Kit as per the manufacturer instructions. After end repair and A-tailing, IDT xGen CS UMI Adapters were ligated and subsequently PCR amplified with IDT xGen UDI Primer pairs. Library quantification was carried out via NanoDrop, and each library was run on an ethidium bromide gel to confirm success. Purified samples libraries were multiplexed to obtain single pools with a combined mass of 2 µg. Library pools were hybridized to a custom-designed KAPA HyperChoice probe set. This probe set captures coding regions of 73 prostate cancer relevant genes, and also introns and flanking regions of selected genes including *TP53*, *PTEN*, and *RB1* (these non-coding regions improve structural rearrangement detection and help inform loss of heterozygosity analysis). The probe set also includes a low-pass whole-genome backbone of regularly spaced probes capturing heterozygous germline SNPs at common frequencies across various ancestral backgrounds. The backbone aids in ctDNA purity estimation and improving chromosome arm copy number calls. The KAPA HyperCap Workflow protocol was followed for hybridization and subsequent wash, recovery, and amplification of the capture regions. Final

libraries were purified with KAPA HyperPure Beads prior to quantification with the Quantus Fluorometer. Pools were diluted to 4 nM and were sequenced on Illumina machines.

Identification of mutations, structural rearrangements and copy number changes

First somatic mutations (single-nucleotide variants and indels) were identified in the targeted sequencing data according to the previously described and validated method [1,2]. In short, at least 8 supporting reads and a variant allele fraction (VAF) of at least 0.5% was required for independent mutation calling. The minimum of 8 supporting reads ensures false positive variant detection due to background error to be very low. As our assay aims for 1500x depth, 8 variant reads out of 1500 equates to a VAF of ~0.5%. This detection threshold is similar to most current commercial pan-cancer liquid biopsy platforms (e.g., FoundationOne Liquid CDx, Guardant360 CDx) [3,4]. Additionally, the observed VAF was required to be at least 20 times higher the average allele fraction from 83 WBC samples and 3 times higher compared to the patient-specific WBC sample again ensuring minimal false positive variant detection.. As all patients had two plasma samples available for ctDNA detection, additional dependent mutation calling was applied. For dependent calling, at least 3 supporting reads and a VAF of 0.5% were required to call a mutation in one plasma sample that was independently identified in the other same-patient plasma sample. A detailed description on structural rearrangement detection and copy number variant detection can be found in our previous reports [1,2].

ctDNA fraction estimation

The ctDNA fraction was estimated using 1) somatic autosomal mutations and 2) germline heterozygous SNPs according to published methodology [1,2], and is described below. The mutation-based ctDNA fraction was calculated using the variant allele fraction (VAF; corrected for statistical outliers and potential loss of heterozygosity; LOH) of autosomal somatic mutations in non-amplified genes (log-ratio <0.3) as detected by the 73-gene panel. Because mutant allele fractions are elevated when a mutation is concurrent with the loss of the other wildtype allele (i.e. LOH), and may be undetectable at low ctDNA fractions, we conservatively assumed that all somatic mutations may be associated with LOH. In regions of LOH, ctDNA fraction and VAF are related as ctDNA% = 2/(1/VAF + 1). To correct for outliers, we modeled the mutant read counts as arising from a binomial distribution, and conservatively calculated what the true VAF would be if the highest observed VAF was a 95% quantile outlier. A ctDNA fraction estimate was calculated for each somatic mutation, and the highest estimate was used as the overall estimate for the sample under the assumption that this mutation was the most likely to be truncal to the metastatic lineage.

Germline variants, sequencing and alignment artifacts, and clonal-hematopoiesis of indeterminate potential (CHIP) can confound somatic mutation-based estimation of ctDNA fraction. These potential confounders are largely eliminated through our parallel sequencing of patient-matched WBC DNA.

We applied an orthogonal copy number-based ctDNA fraction estimation method using germline heterozygous SNPs with allele fractions that deviated from 50% heterozygosity in genes with evidence of LOH. Germline SNPs were identified from paired WBC DNA samples as any variant present in the ExAC or Kaviar databases with a minimum of 75x normal coverage. We filtered for heterozygous intragenic SNPs located on genes that had evidence for a single-copy deletion (log-ratio between -0.3 to -1.0) and contained at least 4 unique SNPs. We calculated the median major allele frequency (|0.5 - VAF| + 0.5) of SNPs within each eligible gene and used this value to estimate ctDNA% = 2 - VAF-1.

To validate our mutation- and copy number-based ctDNA fraction estimations, we leveraged the low-pass whole-genome backbone of heterozygous germline SNPs in our sequencing panel. Models testing various ctDNA fractions and diploid level log ratios were manually fitted to the genome-wide copy number levels and heterozygous SNP allele fractions [5]. Models that most closely adhered to the expected SNP allele fractions for each observed copy number were used to estimate ctDNA%. Samples with low ctDNA fraction (generally <20%) or highly complex copy number profiles due to aneuploidy or subclonality prevented confident ctDNA fraction estimation with this method and thus did not have models fit.

In the case that only an AR amplification was present in the ctDNA and was detected by both the genome-wide SNP backbone and intragenic copy number of the deep targeted sequencing, ctDNA estimation was conservatively estimated at 5%. Our threshold for the detection of mutations was a VAF of 0.5%. After correcting for sampling error and loss of heterozygosity this corresponds to a limit of detection of approximately 1% ctDNA. Therefore, we classified plasma samples into undetected (ctDNA < 1%) and detected (ctDNA ≥ 1%). Similar ctDNA estimate methods were used per patient at both timepoint to compare ctDNA% change.

## References


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