

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
☒	☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	Up to 30 ng of cfDNA, extracted from approximately 4 mL of plasma, was partitioned based on its methylation state, then repaired, ligated with custom molecular barcodes, and amplified using polymerase chain reaction (PCR). The libraries were enriched for a significant portion (~15 Mb) of the DNA methylome, covering over 20,000 epigenomic regions that are differentially methylated in cancer, along with methylation control regions.^{18,23,26,27} The enriched libraries were sequenced using the Illumina NovaSeq 6000 platform. Sequencing data were analyzed with Guardant Reveal using Guardant Health's proprietary bioinformatics pipeline for ctDNA detection based on quantification on predefined epigenomic signals. The bioinformatics algorithm classified each sample as either ctDNA detected (positive) or not detected (negative) according to a predefined statistical likelihood threshold.^{18,23,26,27} A tumor fraction (TF), based on methylation (referred to as mTF), was estimated by normalizing molecule counts in cancer-specific differentially methylated regions (DMRs) against matched control regions within each sample. mTF was calculated at each time point by averaging the cancer-specific DMRs with the highest scores. The limit of detection (LoD=0.005% in advanced breast cancer), limit of quantification (LoQ=0.01%), and accuracy of the mTF were previously established in a separate set of breast, lung, colorectal, and other cancer samples and approved by CLIA (Clinical Laboratory Improvement Amendments). Up to 30 ng of cfDNA, extracted from approximately 4 mL of plasma, then repaired, ligated with custom molecular barcodes, and amplified using PCR. The enriched libraries were sequenced using the Illumina NovaSeq 6000 platform. Genomic variants in over 700 genes were assessed from ctDNA using Guardant360 Liquid, including SNVs/indels, gene-level copy number data, rearrangements, and were generated using a proprietary bioinformatics pipeline (Guardant Health). The maximum variant allele fraction (maxVAF), representing the non-synonymous somatic variant with the highest copy number corrected variant allele fraction, was used as a surrogate for tumor fraction for the genotyping portion of the assay. Somatic, germline, and CHIP status were annotated by the laboratory and were used as such for all genomic analyses. CHIP annotation was performed using a proprietary bioinformatics algorithm assessing variant-level metrics related to
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fragmentomics, methylation patterns, and variant allele frequencies, as well as metadata features aggregated from over 250,000 clinical samples and public databases such as COSMIC and GnomAD.

Data analysis

Statistical analyses were performed using PRISM GraphPad (v9.0) and R version 4.1.0 (R Foundation for Statistical Computing).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

The processed cohort-level clinical and ctDNA data generated in this study are provided in the Supplementary Information and Source Data files. The raw methylation and targeted sequencing data are not available due to proprietary sequencing technologies, patient privacy protections, and institutional data governance policies for small, single-institution cohorts. Individual patient-level clinical data are not publicly available to protect participant confidentiality given the small cohort size, but de-identified summary data are included in the manuscript. Source data are provided with this paper.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

Self identified sex was reported.

Reporting on race, ethnicity, or other socially relevant groupings

Not applicable.

Population characteristics

Patients diagnosed with ER+/HER2- mBC receiving primary treatment at Princess Margaret Cancer Centre were enrolled prospectively from 2018 onwards. Patients were approached by their treating clinicians to participate in the study which may have lead to selection bias.

Recruitment

Participants receiving care at the Princess Margaret Cancer Centre were recruited to the study.

Ethics oversight

Participants provided informed consent for serial blood collection at the time of their standard-of-care blood work, in accordance with the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS2) and approved by the University Health Network (UHN) Research Ethics Board (REB#: 23-5123).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

There were no calculations for sample size. Participants with available data at the time of primary analysis were included.

Data exclusions

One patient was excluded as they did not proceed onto CDK4/6i.

Replication

Replication is not possible as each plasma time point is from a single patient.

Randomization

Participants were not randomized. This was a single arm, prospectively collected, retrospectively analyzed cohort.

Blinding

Data was analyzed retrospectively and participants, clinicians, and investigators were blinded to clinical outcomes which were collected after sequencing results were obtained.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	NCT03702309
Study protocol	Protocol available upon request.
Data collection	Data was extracted from the electronic health record in accordance with the approved REB protocol.
Outcomes	Predefined analyses included assessment of baseline rate of ctDNA detection (clinical sensitivity), evaluation of ctDNA detection through each participant's clinical course, and exploratory characterization of the prognostic association with ctDNA detection and clinical outcomes. Concordance between concurrent matched liquid biopsy timepoints and radiographic assessments were considered if assessments performed within 30 days of each other. Definitions of ctDNA response and progression are outlined in the manuscript. These were not predefined and should be considered exploratory.

Plants

Seed stocks	Not applicable.
Novel plant genotypes	Not applicable.
Authentication	Not applicable.