Serial ctDNA Monitoring in Metastatic Breast Cancer Using an Ultrasensitive Tumor-informed Structural Variant-based Assay

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INTRODUCTION

- Standard ctDNA assays (e.g., targeted panels, methylation) typically detect variant allele fractions down to ~0.1-0.5%, while current MRD-grade tumor-informed SNV panels often reach an level of detection 95% (LoD95) around ~0.01-0.001% VAF, with further sensitivity requiring 100's-1000's of variants.[1,2]
- Improved analytical sensitivity improves the rate of ctDNA detection in early-stage breast cancer (EBC) and may improve detection in metastatic breast cancer (mBC), potentially enabling finer resolution of on-treatment ctDNA dynamics.[1-4]
- Tumor-informed, multiplex dPCR tracking of tumor-specific structural variants (SVs) achieves ultrasensitive detection (LoD95: <0.001% VAF). SVs provide near-zero background noise compared to SNVs, improving specificity for tumor signal quantification.[3]
- SV fingerprints may also be generated directly from plasma using deep cfDNA whole genome sequencing (WGS), offering a path to tissue-free fingerprint generation when archival tumor is unavailable. Using SVs for this approach rather than SNVs circumvents the issues associated with CHIP and technical noise, which can be problematic at low ctDNA fractions.
- Ultrasensitive SV-based tracking may better resolve early molecular response and impending progression in mBC.
- Longitudinal ctDNA dynamics across successive lines of therapy within the same mBC patients remain poorly understood, and their association with radiographic response, duration of benefit, and progression is not well defined.

METHODS

- Patients with mBC (all receptor subtypes) receiving systemic therapy were prospectively enrolled (cross-sectionally) at the Princess Margaret Cancer Centre (LIBERATE; B-FIRST: Blood biomarker Fully Integrated Response assessment and Surveillance of Treatment; **Figure 1A**) cohort (NCT03702309).
- Clinical variables, including tumor stage, receptor status, treatment regimens, radiographic outcomes (radiologist adjudicated, RECIST), treatment discontinuation, and death were extracted from medical records.
- The primary endpoint was concordance between ctDNA dynamics and clinical outcome using exploratory definitions of ctDNA response and progression (sensitivity, SN; specificity, SP; positive predictive value, PPV; negative predictive value, NPV)
- FFPE tumor tissue from primary or metastatic lesions was processed for WGS and the presence of ctDNA was evaluated on all available plasma timepoints using the Pathlight™ Assay (SAGA Dx, Morrisville, NC). Tissue-free SV-based fingerprints were generated from deep cfDNA WGS in select patients without available baseline tissue, but with a blood sample available at enrolment, prior to initiation of treatment or at clinical progression prior to initiation of next-line therapy (research use only, RUO).
- Tumor-specific SVs were identified in plasma cfDNA via multiplex dPCR, starting with SV fingerprint generation from tumor-only WGS (**Figure 1B**). Candidate SVs were orthogonally validated on tumor DNA by targeted dPCR, with buffy coat screening used to exclude germline variants and clonal hematopoiesis (CHIP) artifacts, yielding validated panels of up to 16 somatic SVs per patient. When calling SVs *de novo* from cfDNA, orthogonal validation was performed by dPCR using NGS libraries as a positive control and matched and unmatched negative controls.
- Clinical and radiographic outcomes were last updated on April 1st, 2026.

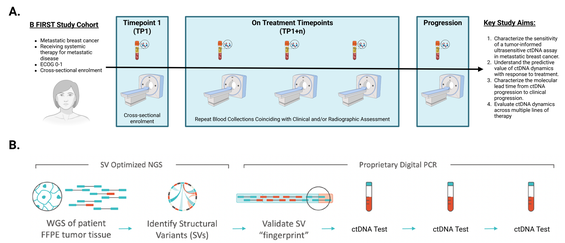

Figure 1. Study Overview. (A) Princess Margaret Cancer Centre cohort overview, including targeted plasma collection timepoints. (B) Workflow of the Pathlight assay. WGS is performed on DNA from FFPE tumor tissue, followed by identification and ranking of somatic SVs. Orthogonal validation of selected SVs is then performed on remaining tumor DNA. Personalized ctDNA panels containing up to 16 somatic SVs are subsequently assessed on cfDNA to detect and quantify ctDNA ("ctDNA test").

RESULTS

- 73 patients with available tumor tissue and ≥2 plasma timepoints were selected for retrospective ctDNA analysis
- Panels were successfully generated from 66/73 patients (86%) **(Table 1, Figure 2)**
  - FFPE (tissue) derived fingerprints: 61/64 (95%); 3/64 (5%) with <4 SVs
  - 9 patients did not have sufficient DNA for fingerprint generation and deep cfDNA WGS was performed to generate panels
    - cfDNA WGS derived fingerprints were successful (>4 SVs) in 5/9 (56%)
- Median number of SVs per fingerprint: 13 (range: 4-16)
- 380 plasma timepoints were evaluated (median: 5 per patient; range: 2-19)
  - Detectable: 294/380 (77%)
  - Undetectable: 85/380 (22%)
    - Patients with ongoing exceptional response enrolled cross-sectionally (n=13; 7/78 timepoints with detectable ctDNA, which cleared on treatment) across all subtypes
  - Failed: 1/380 (<1%)
- Median tumor genome copies/mL (TGM) in detectable timepoints: 13.6 TGM (range: 0.0021-428755 TGM)
  - Below 1 TGM: 89/294 (30%)
  - Below 0.1 TGM: 8/294 (2%)

	All Participants (n=66) (%)
Age at Study Enrolment, Median (range)	59 (33-85)
Sex, n (%)	
Female	66 (100%)
Menopausal Status	
Pre	18 (27%)
Post	48 (73%)
Survival Status	
Living	58 (88%)
Deceased	8 (12%)
Receptor Subtype	
ER+/HER2-	41 (62%)
TNBC	9 (14%)
HER2+	16 (24%)
Treatment At Enrolment	
Endocrine Therapy (+/-Targeted Therapy)	29 (44%)
Cytotoxic Therapy	32 (48%)
Targeted Therapy*	3 (5%)
Clinical Trial (Cytotoxic)	2 (3%)
Treatment Line at Enrolment, Median (range)	1 (1-9)

Table 1: Baseline Participant Characteristics. *not including CDK4/6i

A.

Patient ID	Subtype	Estimated ctDNA% in Sequenced Timepoint	Sequencing Depth	Timepoint of Blood Collection	Successful Tissue-free Fingerprint	Final Number of Validated SVs
1	HER2+	<0.1%	~250X	At treatment progression	No	NA
2	ER+/HER2-	<0.1%	~250X	At treatment progression	No	NA
3	ER+/HER2-	0.02%	~325X	At treatment progression	No	NA
4	ER+/HER2-	0.05%	~325X	At treatment progression	No	NA
5	ER+/HER2-	>10%	~250X	At treatment progression	Yes	14
6	ER+/HER2-	>10%	~250X	At treatment progression	Yes	16
7	ER+/HER2-	1-5%	~250X	At treatment progression	Yes	14
8	ER+/HER2-	0.1-1%	~325X	At stage IV diagnosis	Yes	7
9	ER+/HER2-	>10%	~225x	At stage IV diagnosis	Yes	16

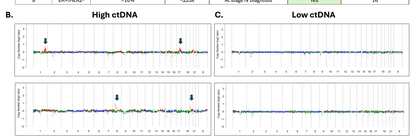

Figure 2. Tissue-Free ctDNA Fingerprint Generation from Deep cfDNA Whole-Genome Sequencing. (A) Summary of nine patients undergoing tissue-free fingerprint generation from cfDNA WGS (~225-325x depth). Successful fingerprint generation (green) was achieved in patients with sufficient ctDNA tumor fraction (≥0.1%), while attempts failed (orange) in samples with tumor fractions below this threshold. (B and C) Representative genome-wide copy number profiles (log2 ratio, plotted by chromosome) from cfDNA WGS. Left panels show successful cases with detectable focal copy number alterations (arrows) supporting SV calling and fingerprint generation, while right panels show examples of failed cases with flat profiles consistent with low ctDNA content.

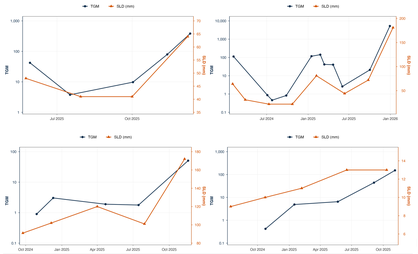

Figure 3. Concordance Between ctDNA Dynamics and Radiographic Tumor Burden. Representative, RECIST-evaluable, individual patients showing paired longitudinal ctDNA dynamics (TGM, navy, left y-axis, log scale) and radiographic sum of longest diameters (SLD, mm, orange, right y-axis). Each panel depicts a single patient. Concordant rises in ctDNA and SLD illustrate the correspondence between molecular and radiographic measures of disease burden.

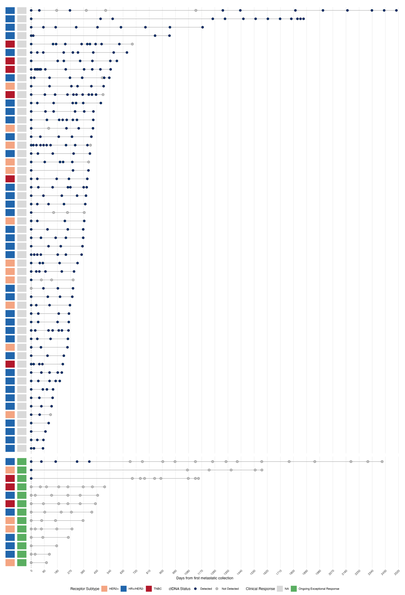

Figure 4. Longitudinal ctDNA Sampling Across the Cohort. Representative swimmer plots in the cohort of 66 patients. Each row represents an individual patient and each dot represents a ctDNA timepoint. The x-axis indicates days from first metastatic plasma collection. Patients are stratified into two groups: all patients (top) and those with an ongoing exceptional response (bottom). Patients were enrolled cross-sectionally so pre-treatment timepoints are not available for some patients. Left annotation bars indicate receptor subtype (orange: HER2+, blue: ER+/HER2−, red: TNBC) and clinical response status (grey: not applicable, green: ongoing exceptional response). ctDNA status at each timepoint is shown as detected (navy) or not detected (grey).

RESULTS

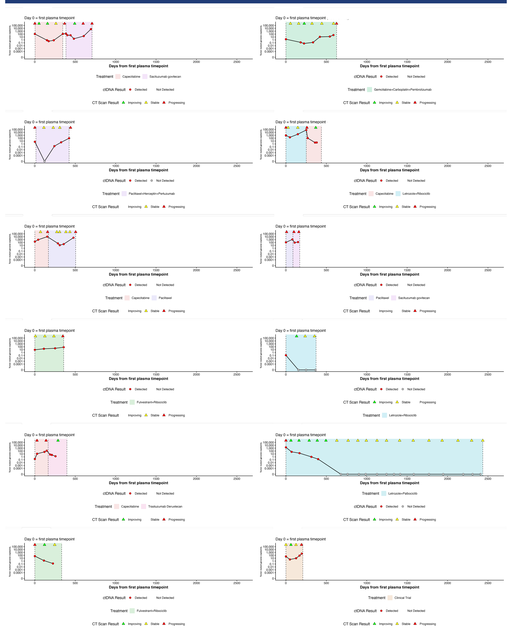

Figure 5. Longitudinal ctDNA Dynamics and Treatment Timelines. Representative treatment timelines and ctDNA dynamics are shown for individual participants. The x-axis indicates days from first plasma collection and the y-axis shows ctDNA quantification (mutant genome equivalents/mL, TGM, log scale). Red circles indicate detectable ctDNA and grey circles indicate not detected. Shaded regions denote lines of systemic therapy, color-coded by regimen as labeled in each panel. Triangles mark CT scan results per radiologist-adjudicated outcomes (green: improving, yellow: stable, red: progressing).

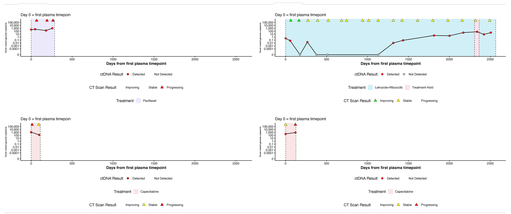

Figure 6. Longitudinal ctDNA Dynamics and Treatment Timelines for Patients with Tissue-free Fingerprints. Representative treatment timelines and ctDNA dynamics are shown for individual participants whose SV fingerprints were generated from deep cfDNA WGS. The x-axis indicates days from first plasma collection and the y-axis shows ctDNA quantification. Red circles indicate detectable ctDNA and grey circles indicate not detected. Shaded regions denote lines of systemic therapy, color-coded by regimen as labeled in each panel. Triangles mark CT scan results per radiologist-adjudicated outcomes (green: improving, yellow: stable, red: progressing).

RESULTS

A.

LB-RECIST Call	ctDNA Rise Radiographic Progression	ctDNA Decrease Stable or Improving Disease	ctDNA Rise Stable or Improving Disease	ctDNA Decrease Radiographic Progression
CPD	44	1	63	0
CSD	1	4	1	0
CPR	0	64	0	3
CCR	0	68	0	0

B.

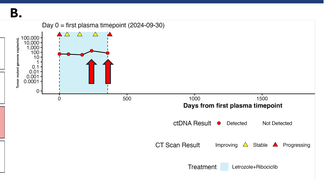

Figure 7. ctDNA-based LB-RECIST Concordance with Radiographic Response. (A) Confusion matrix of 249 paired ctDNA–radiographic timepoints from this cohort. Liquid Biopsy (LB)-RECIST categories were adapted from Gouda et al.[4] (*Annals of Oncology 2024*) and applied to the ctDNA dynamics in this cohort, comparing each timepoint to the immediately preceding ctDNA timepoint using a ±10% threshold: ctDNA Complete Response (CCR) (prev > 0 and curr = 0 or curr = 0 and prolonged response: clearance to undetectable); ctDNA Partial Response (CPR) ((curr − prev)/prev < −10%); ctDNA Stable Disease (CSD) ((|curr − prev|/prev| ≤ 10%); and ctDNA Progressive Disease (CPD) ((curr − prev)/prev > +10%, or emergence from undetectable). CCR, CPR, and CSD were classified as molecular response and CPD as non-response; CND was excluded as it is subsumed within CCR as all patients had exceptional and durable responses to therapy. Applied to this cohort, LB-RECIST achieved 94% sensitivity and 98% negative predictive value for radiographic progression. (B) Representative patient on letrozole + ribociclib in whom two successive rises in ctDNA (red arrows) from nadir preceded radiographically confirmed progression, illustrating the molecular lead-time captured by LB-RECIST. The second arrow illustrates a sample with lower ctDNA level from previous, highlighting the potential impact of cyclical therapies and the timing of collections.

CONCLUSIONS

- Tumor-informed, multiplex dPCR SV-based ctDNA detection enabled ultrasensitive longitudinal monitoring in mBC across multiple lines of treatment
- Tissue-free fingerprint generation from deep cfDNA WGS is feasible in patients with sufficient circulating tumor content
- ctDNA dynamics tracked closely with radiographic tumor burden
- Ongoing enrollment in B-FIRST will expand this cohort to develop a blood-based response monitoring framework

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UHN Princess Margaret Cancer Biobank
Canadian Cancer Society
Société canadienne du cancer

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