Longitudinal ctDNA Tracking in Early and Recurrent Breast Cancer Using an Ultrasensitive Structural Variant-Based Assay: An Updated Analysis from the TRACER Study

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INTRODUCTION

- Detection of circulating tumor DNA (ctDNA) after curative-intent therapy in early-stage breast cancer (EBC) is strongly prognostic for disease recurrence.[1,2]
- We previously reported an ultrasensitive structural variant (SV) and digital PCR (dPCR) ctDNA assay (Pathlight™, SAGA Dx) in 100 patients with EBC treated with neoadjuvant therapy, demonstrating high sensitivity, specificity, and long lead times to distant relapse.[3]
- This personalized SV-based fingerprint enables ctDNA detection at ultrasensitive levels (LoD95 = 5.2 PPM) and offers low technical error rates without requiring deep sequencing.[3]
- It is unknown whether this SV-dPCR approach can effectively monitor ctDNA dynamics in the metastatic setting, and whether the underlying SV breakpoints remain stable under treatment pressure and clonal evolution.
- We now report findings from an expanded EBC cohort with extended follow-up and retrospective application of the same personalized SV-dPCR fingerprints to ctDNA monitoring in patients who developed metastatic recurrence.

METHODS

- Patients with EBC including ER+/HER2-, TNBC, and HER2+ subtypes were prospectively enrolled in the LIBERATE neoadjuvant therapy cohort (TRACER; NCT03702309) at the Princess Margaret Cancer Centre (Toronto, Canada).
- The Pathlight assay (SAGA Dx, Morrisville, NC) detects tumor-specific SVs in plasma cfDNA via multiplex dPCR, starting with SV fingerprint generation from tumor-only WGS. An orthogonal validation step excludes germline and clonal hematopoiesis artifacts using buffy coat and confirms panels of up to 16 somatic SVs using targeted dPCR on the tumor DNA.
- Clinical variables including tumor stage, receptor status, and treatment regimens were abstracted from medical records. The primary endpoint was distant recurrence-free interval (DRFI), and lead time was defined as the interval between first post-surgical ctDNA detection and clinically confirmed recurrence.
- Patients who developed metastatic recurrence were subsequently consented into a follow-up study evaluating ctDNA dynamics in the metastatic setting. Plasma was collected prospectively at the time of radiographic restaging and analyzed retrospectively using the same tumor-informed SV-dPCR fingerprint to quantify ctDNA levels. Clinical data including metastatic sites, systemic therapies, real-world progression-free survival (rwPFS), treatment lines, radiographic response, and overall survival (OS) were extracted from the medical record.
- All clinical data including recurrence outcomes were updated on November 20, 2025.

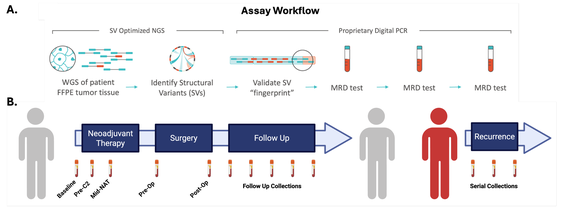

Figure 1. Study Overview. Analytical validity of the locked Pathlight ctDNA Assay workflow was evaluated. **(A)** Workflow of the Pathlight Assay. WGS is performed on DNA from FFPE tumor tissue followed by identification and ranking of somatic SVs. Orthogonal validation of selected SVs is performed on remaining tumor DNA. Personalized ctDNA panels with up to 16 somatic SVs are assessed on ctDNA to identify ctDNA ("MRD test"). **(B)** Princess Margaret Cancer Centre cohort overview including targeted plasma collection timepoints. Many patients who experienced a recurrence were consented to continue prospective plasma collection in the metastatic setting.

RESULTS

- 121 patients (ER+/HER2- n=42; TNBC n=34; HER2+=45) with 723 timepoints were analyzed.
- Median follow up was 4.2 years (range: 0.5-8.8 years):
  - ER+/HER2- = 4.2 years (range: 0.6-8.8 years)
  - TNBC = 3.3 years (range: 0.5-8.7 years)
  - HER2+ = 4.9 years (range: 1.6-8.0 years)
- ctDNA was detected in 95% (105/111) of baseline clinical samples, with a median VAF of 0.16% (range: 0.0011–38.7%):
  - ER+/HER2- = 90% (35/39)
  - TNBC = 96% (27/28)
  - HER2+ = 98% (43/44)
- ctDNA was identified in 316/723 (44%) of all analyzed samples with a VAF range of 0.00003-38.7%, including 126/316 (40%) with VAF <0.01% (100 PPM) and 49/316 (16%) with VAF <0.001% (10 PPM).

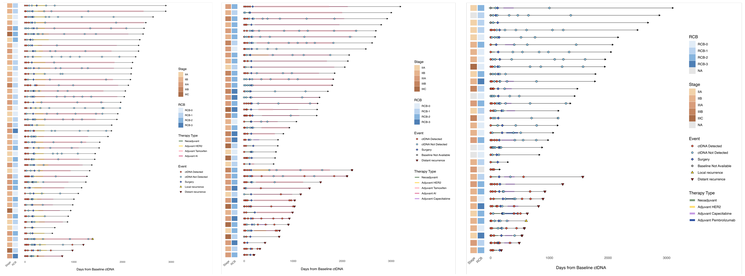

Figure 2. Swimmer Plots and Clinical Events. Swimmer plots show treatment courses, clinical events, and serial ctDNA results for (A) ER+, (B) TNBC, and (C) HER2+ early breast cancer. Each bar represents one patient from baseline through neoadjuvant therapy, surgery, adjuvant treatment, and follow-up. Symbols indicate ctDNA detection, surgery, and sites of recurrence; Stage and RCB class are shown on the left. Across all subtypes, ctDNA rose well before clinical relapse, with a **median lead time of 334 days** (range: 0–1931). **21/22 patients (95%)** had ctDNA detected prior to or at recurrence; one patient remained ctDNA-negative, with a last negative result **938 days before relapse**. RCB = residual cancer burden; AI = aromatase inhibitor.

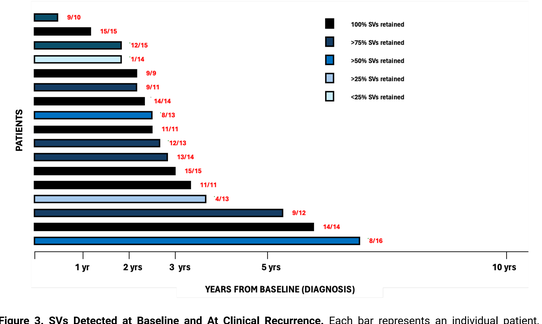

Figure 3. SVs Detected at Baseline and At Clinical Recurrence. Each bar represents an individual patient, illustrating the proportion of baseline tumor-specific SVs that remain detectable in plasma at the time of clinical recurrence. Bar length indicates the time from baseline diagnosis to recurrence. Colors denote the percentage of baseline SVs with retained detection at recurrence. **SV retention is 79% at recurrence.** Excludes cases <0.08% VAF where individual SV detection would be susceptible to stochasticity.

	Patients (n=121)
Age	49 (24-79)
Clinical Stage	n, (%)
I	4 (3.3)
IIA	23 (19.0)
IIB	42 (34.7)
IIIA	32 (26.4)
IIIB	10 (8.3)
IIIC	10 (8.3)
Receptor Subtype	n, (%)
ER+/HER2-	42 (34.7)
HER2+	45 (37.2)
TNBC	34 (28.1)
Tumor Grade	n, (%)
2	31 (25.6)
2 to 3	15 (12.4)
3	75 (62.0)
Residual Cancer Burden	n, (%)
RCB-0	32 (26.4)
RCB-1	34 (28.1)
RCB-2	40 (33.1)
RCB-3	14 (11.6)

Table 1: Baseline Participant Characteristics in the TRACER Cohort.

RESULTS

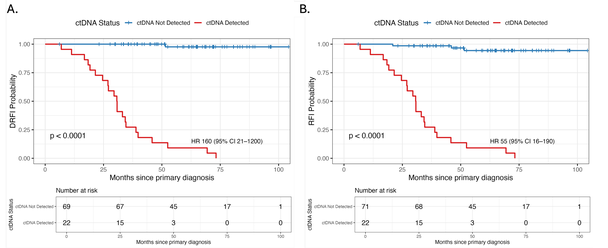

Figure 4. ctDNA Detection is Highly Prognostic of Recurrence in Early-stage Breast Cancer. (A) Distant recurrence-free interval (DRFI) by ctDNA status including all patients who had adjuvant sampling with ctDNA detected. 21/22 patients had ctDNA detected prior to or at clinical recurrence (1 not detected, last negative 938 days prior). Any ctDNA detection: Sensitivity= 95%, Specificity = 100%, Positive Predictive Value = 100%, Negative Predictive Value = 99% (B) Recurrence-free interval (RFI) by ctDNA status. All recurrences including distant and local included. 2 local recurrences were not detected prior to clinical recurrence.

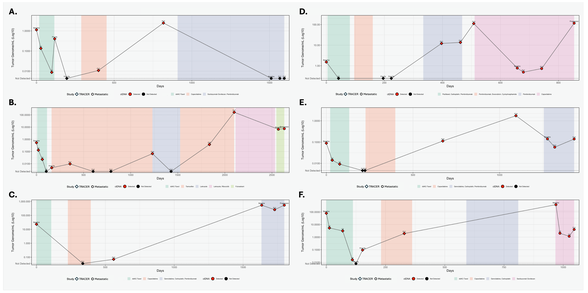

Figure 5. ctDNA Dynamics From Early-stage Breast Cancer Through Metastatic Recurrence. Longitudinal structural-variant digital PCR (SV-dPCR) ctDNA trajectories are shown for six patients (A-F), spanning the early breast cancer (EBC) and metastatic breast cancer (MBC) disease course. Shaded intervals denote systemic therapies across neoadjuvant, adjuvant, and metastatic settings. In the EBC phase (left of each panel), ctDNA frequently became undetectable following initial therapy, corresponding to clinical response. At metastatic recurrence, the same personalized SV fingerprints were reapplied to monitor ctDNA in the advanced-disease setting. Rising ctDNA levels anticipated radiographic progression, while declines aligned with treatment response. These cases illustrate the ability of SV-dPCR ctDNA monitoring from curative-intent treatment through metastatic relapse. (A) Patient 1 - complete response (RECIST 1.1) on SG+Pembrolizumab (B) Patient 2 (C) Patient 3 (D) Patient 4 (E) Patient 5 and (F) Patient 6.

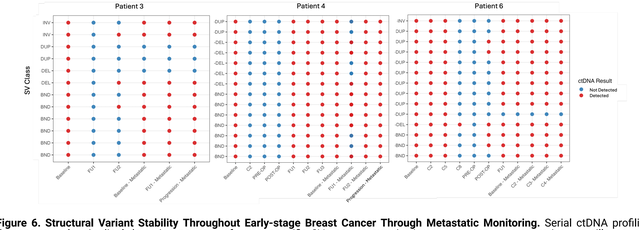

Figure 6. Structural Variant Stability Throughout Early-stage Breast Cancer Through Metastatic Monitoring. Serial ctDNA profiling demonstrates longitudinal detection patterns of tumor-specific SVs across curative-intent treatment, postoperative surveillance, and metastatic progression. Each panel represents an individual patient, showing SV class (BND, DEL, DUP, INV) over time. Red points indicate SVs detected in plasma and blue points indicate those not detected. Across patients, most SVs show stable, persistent detection, with recurrence often preceded by re-emergence of multiple SVs. These findings highlight the biological stability of SV fingerprints and support their use for longitudinal ctDNA monitoring. BND: Break End, DEL: Deletion, DUP: Duplication, INV: Inversion.

RESULTS

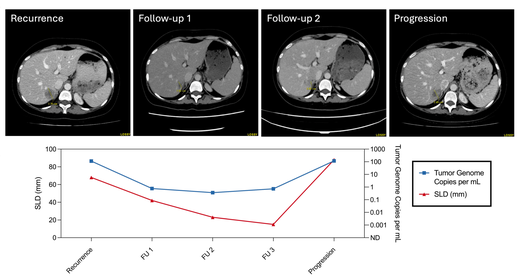

Figure 7. Radiographic and Molecular Dynamics During Treatment Response and Progression. Representative axial images of serial CT scans of the abdomen illustrating the evolution of a hepatic metastasis from initial recurrence through follow-up assessments and eventual progression. Corresponding ctDNA trajectories are illustrated showing ctDNA rise anticipating radiographic progression. The patient experienced target and non-target PD per RECIST 1.1 criteria. SLD = Sum of the Longest Diameters.

CONCLUSIONS

- The Pathlight assay detects ctDNA before clinical recurrence across all EBC receptor subtypes.
- Tumor-specific SVs remain detectable throughout curative-intent and metastatic therapy, indicating breakpoint stability despite treatment pressure.
- Early data show that this approach supports consistent longitudinal ctDNA monitoring in the recurrent metastatic setting, regardless of systemic therapy.
- Prospective studies are needed to establish clinical utility.

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