Somatic Structural Variation in Breast Cancer and its Application in Longitudinal Analysis of Circulating Tumor DNA in Early Breast Cancer

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INTRODUCTION

- Detection of circulating tumor DNA (ctDNA) following curative-intent therapy in early breast cancer (EBC) is prognostic of disease recurrence.[1-4]
- Many tumor-informed ctDNA assays leverage multiple tumor-specific single nucleotide variants (SNVs) through multiplex PCR or next-generation sequencing (NGS). These assays have achieved high sensitivity and specificity in ctDNA detection, with a Limit of Detection (LoD95) between 0.01-0.001% (100-10 parts per million, PPM). [1-4]
- Monitoring of ctDNA at an ultrasensitive level (LoD95<0.001%, less than 10 PPM) can be achieved by tracking hundreds to thousands of tumor-specific SNVs, but the ability to reach this level of sensitivity by tracking other common genomic alterations is unknown. [5]
- Structural variants (SVs), such as translocations, inversions, tandem duplications and large deletions, identifiable through whole genome sequencing (WGS), are distinct hallmarks of cancer that contribute to genomic instability and may reflect unique aspects of tumor biology. The resulting DNA breakpoints are tumor-specific and resistant to certain PCR and sequencing errors.
- Digital PCR (dPCR) offers a promising approach for detecting tumor-specific SVs in ctDNA. Compared to NGS, dPCR avoids the need for high sequencing depths, reducing error rates and providing operational advantages in detecting ctDNA in the presence of high levels of normal cell-free DNA (cfDNA).
- The complete landscape of SVs in breast cancer and their potential use for ctDNA detection at an ultrasensitive level (<10 PPM) remains largely unexplored.

METHODS

- Paired tumor-normal WGS data from 16,247 cancer patients in the Genomics England 100,000 Genomes Project were used to generate somatic SV calls, removing artifacts with additional germline SV data. Somatic SVs were filtered based on size, breakpoint homology, and location. Breast cancer-specific analyses focused on a subset of 1,180 cases, and was used to assess SV burden differences across breast cancer subtypes.[6]
- 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.
- The LoD95 was determined using the probit method for a standard cfDNA input amount (70 ng, NA24385, Coriell DNA, fragmented to mimic cfDNA) using a BT-474 (HER2+ breast cancer cell line) with a dilution range from 0.0005% (5 PPM) to 0.00004% (0.4 PPM) tumor fraction. External clinical validation was performed using a cohort of patients with EBC.
- Patients with EBC (all subtypes) receiving neoadjuvant therapy (NAT) were enrolled from the Princess Margaret Cancer Centre (LIBERATE) cohort (NCT03702309). FFPE tumor tissue was processed for WGS and the presence of ctDNA was evaluated on all available plasma timepoints.
- Clinical variables, including tumor stage, receptor status, and treatment regimens were extracted from medical records. The primary endpoint, distant recurrence-free interval (DRFI), was evaluated. The lead-time to recurrence was calculated as first ctDNA detection after completion of surgery to confirmed clinical recurrence.
- Recurrence outcomes were last updated on September 30, 2024.

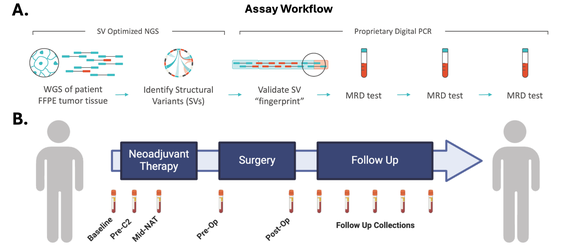


Figure 1. Study Overview. Analytical validity of the locked Pathlight ctDNA Assay workflow was evaluated. **(A)** Workflow of the Pathlight Assay. WGS is performed on DNA from FFPE tumor tissue followed by identification and ranking of somatic SVs. Orthogonal validation of selected SVs is performed on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

RESULTS

- Overall, 1,292,794 eligible SVs were detected across the 16,247 tumors evaluated, with SV burden varying significantly among cancer types in the Genomics England data set **(Fig. 2A)**.
- Notably high SV burdens were observed in upper gastrointestinal tumors (median SV burden=85, n=265), sarcoma (median SV burden=80, n=1819), ovarian cancer (median SV burden=78, n=634), and breast cancer (median SV burden=60, n=3009).
- Within breast cancer cases, a significantly higher SV burden was observed in HER2+ and TNBC compared to ER+ (p<0.0001) **(Fig. 2B)**
- The LoD95 of the Pathlight assay was 0.00052% **(5 PPM, Fig. 3A)** with variants detected as low as 4 in 10 million (0.00004% or 0.4 PPM) **(Fig. 3B)**
- ctDNA was detected in **96%** (91/95) of **baseline clinical samples**, with a median VAF of 0.15% (range: 0.0011−38.7%) **(Fig. 4A)**.
- Baseline ctDNA detection was similar across clinical receptor subtypes: **TNBC 96.0% (23/24), ER+ 94.0% (30/32), and HER2+ 97.4% (38/39)**
- ctDNA was identified in 260/568 (46.0%) of all analyzed samples with a VAF range of 0.00006-38.7%, including 97/260 (37.5%) with VAF <0.01% (100 PPM) and 34/260 (13.1%) with VAF <0.001% (10 PPM).

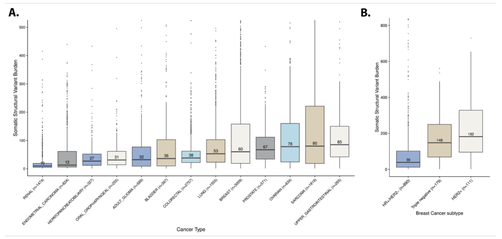
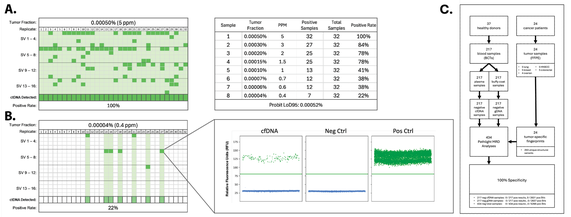


Figure 2. Structural Variant Burden Across Solid Tumors and Within Breast Cancer Subtypes. (A) Somatic structural variant burden across adult solid tumor types in the UK 100,000 Genomes Project. The median SV burden for each cancer type is noted. Cancer types with <100 samples are not shown. **(B)** Somatic SV burden within breast cancer subtypes, as derived from the UK 100,000 Genomes Project.[6]



Sample	Tumor Fraction	PPM	Positive Samples	Total Samples	Positive Rate
1	0.00050%	5	32	32	100%
2	0.00030%	3	27	32	84%
3	0.00020%	2	25	32	78%
4	0.00015%	1.5	25	32	78%
5	0.00010%	1	13	32	41%
6	0.00007%	0.7	12	32	38%
7	0.00006%	0.6	12	32	38%
8	0.00004%	0.4	7	32	22%

Probit LoD95: 0.00052%

Figure 3. Contrived cfDNA Samples Prepared for Assessment of Assay Analytical Validity. (A) Samples prepared at 8 tumor fractions, from 0.0005% (5 PPM) to 0.00004% (0.4 PPM), were tested using 70 ng of cfDNA input. For each tumor fraction, 32 replicates were analyzed to calculate "positive rates", yielding LoD95 of 0.00052% (5.2 PPM). Individual SV results are presented for the highest and lowest tested tumor fractions, using matrices of replicate number (1 − 32) versus SV number (1 – 16). Dark-green-filled cells indicate positive results. **(B)** Example digital PCR data (1D plots) for one positive SV result, including positive and negative controls. A representative threshold value is illustrated. **(C)** 434 ctDNA analyses were performed using tumor-negative cfDNA and gDNA samples to assess specificity. Assays were generated for 24 tumor FFPE samples from 24 cancer patients, including five cancer types. The 24 assays, including 293 unique SVs, analyzed tumor-negative cfDNA and gDNA samples extracted from plasma and buffy coat, respectively, from healthy donors. All tumor-negative cfDNA (217) and gDNA (217) samples were correctly called (100% specificity).

Cohort Characteristics				
	All Patients (N=100)	Triple Negative (N=25)	ER-Positive (N=53)	HER2-Positive (N=20)
Follow Up, Median (Years) [Range]	5.3 [0.5-7.7]	2.8 [0.5-7.5]	4.9 [0.5-7.7]	3.8 [0.6-7.6]
Age, Median (Range)	50 [24-79]	47.5 [29-68]	52 [31-73]	48 [24-79]
Sex, n (%)				
Female	100 (100%)	25 (100.0%)	53 (100.0%)	20 (100.0%)
Male	0 (0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Menopausal Status, n (%)				
Post	42 (42.0%)	9 (32.0%)	11 (41.5%)	18 (46.2%)
Pre	58 (58.0%)	19 (68.0%)	18 (34.0%)	21 (53.8%)
Tumor Size, n (%)				
T1/T2	65 (65.0%)	20 (71.0%)	17 (31.5%)	28 (71.8%)
T3/T4	35 (35.0%)	8 (29.0%)	36 (68.5%)	11 (28.2%)
Nodal Status, n (%)				
Node-negative	31 (31.0%)	13 (46.0%)	6 (18.2%)	12 (30.8%)
Node-positive	69 (69.0%)	15 (54.0%)	27 (81.8%)	27 (69.2%)
Stage, n (%)				
I	4 (4.0%)	4 (14.3%)	0 (0.0%)	0 (0.0%)
II	59 (59.0%)	18 (64.3%)	13 (39.4%)	28 (71.8%)
III	37 (37.0%)	6 (21.4%)	20 (60.6%)	11 (28.2%)
Grade (Diagnostic biopsy), n (%)				
2	36 (36.0%)	2 (7.0%)	15 (45.5%)	9 (23.1%)
2 to 3	10 (10.0%)	2 (7.0%)	4 (12.1%)	4 (10.3%)
3	64 (64.0%)	24 (86.0%)	14 (42.4%)	26 (66.6%)
Histology, n (%)				
IDC	83 (83.0%)	26 (93.0%)	30 (90.9%)	27 (64.3%)
Other	7 (7.0%)	2 (7.0%)	3 (9.1%)	2 (5.1%)
Neoadjuvant Treatment, n (%)				
Anthracycline + Taxane	83 (83.0%)	25 (89.3%)	31 (94.0%)	27 (69.2%)
Endocrine Therapy	1 (1.0%)	0 (0.0%)	1 (3.0%)	0 (0.0%)
Taxane	5 (5.0%)	3 (11.0%)	1 (3.0%)	1 (2.6%)
Taxane+Platinum	11 (11.0%)	0 (0.0%)	0 (0.0%)	11 (28.2%)
Recurrences, n (%)				
No	76 (76.0%)	19 (67.9%)	21 (63.6%)	36 (92.3%)
Yes (Local)	2 (2.0%)	1 (3.6%)	0 (0.0%)	1 (2.6%)
Yes (Distant)	22 (22.0%)	8 (28.5%)	12 (36.4%)	2 (5.1%)

Table 1: Baseline Participant Characteristics in the EBC Clinical Validation Cohort.

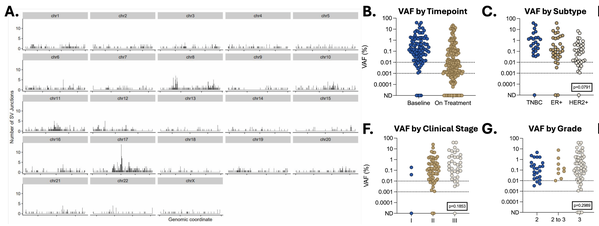


Figure 4. Tumor-specific SV-based Assay Analysis. (A) Histogram of the genomic coordinates of validated genome-wide structural variant breakpoints in the EBC cohort (n=100), using 100,000bp bins. **(B)** Representation of VAF at baseline (n=95) and in all samples (n=568) tested in the EBC cohort. Comparison of baseline VAF and routine clinical variables: **(C)** Receptor Subtype **(D)** Clinical Nodal-status **(E)** Tumor-size **(F)** Clinical Stage **(G)** Nottingham grade (diagnostic biopsy) and **(H)** in participants with and without recurrence. **(I)** Fingerprint copy number in those with and without recurrence. Chr=chromosome, N=nodal status, T=tumor size, VAF=variant allele fraction, CN=copy number.

RESULTS

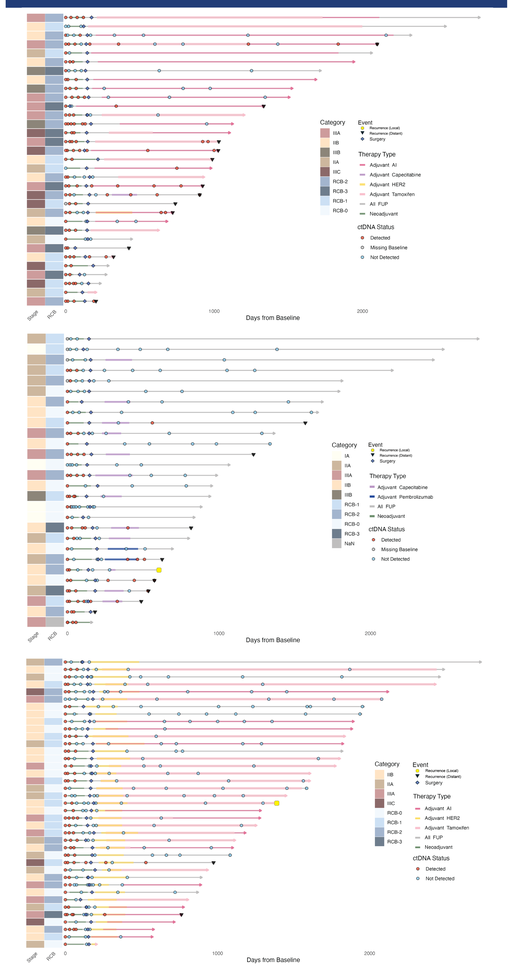


Figure 5. Swimmer Plots and Clinical Events. Participants' clinical and ctDNA analysis timelines for **(A)** ER+ **(B)** TNBC and **(C)** HER2-positive EBC. ctDNA was detected prior to distant recurrence in all cases (100% sensitivity) with a **median lead time of 417 days (range: 4−1931 days).** Stage I, II, III, RCB: residual cancer burden, cN: clinical nodal status, cT: clinical tumor size, AI: aromatase inhibitor.

RESULTS

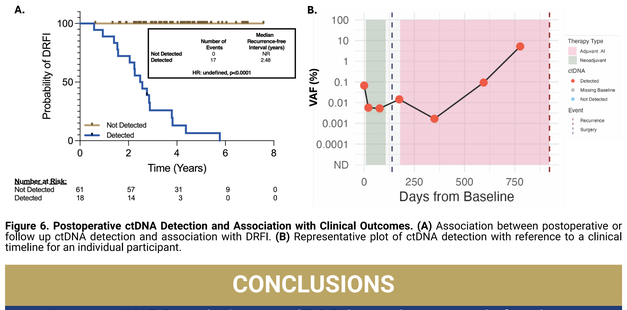


Figure 6. Postoperative ctDNA Detection and Association with Clinical Outcomes. (A) Association between postoperative or follow up ctDNA detection and association with DRFI. **(B)** Representative plot of ctDNA detection with reference to a clinical timeline for an individual participant.

CONCLUSIONS

- A tumor WGS and plasma dPCR-based approach for the identification of ctDNA using patient-specific SVs is feasible and can be deployed in clinically-relevant patient populations.
- The ability to detect ctDNA in nearly all participants at baseline (96% overall), including in 94% of participants with ER+ disease, suggests the assay's high analytic sensitivity is clinically relevant.
- ctDNA was detected in all participants prior to metastatic recurrence (100% sensitivity and specificity) with a median lead time of 417 days (range: 4-1931 days).
- These data should motivate future prospective studies to evaluate ctDNA-guided strategies and study the clinical utility of ctDNA detection.

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CANCER GENOMICS PROGRAM

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REFERENCES

1. Magbanua, M. J. M. *et al.* Circulating tumor DNA in neoadjuvant-treated breast cancer reflects response and survival. *Ann. Oncol.* **32**, 229−239 (2021).
2. Lipsyc-Sharf, M. *et al.* Circulating tumor DNA (ctDNA) and late recurrence in high-risk, hormone receptor−positive, HER2-negative breast cancer (CHRP). *J. Clin. Oncol.* **40**, 103−103 (2022).
3. Coakley, M. *et al.* Abstract PD5-03: PD5-03 Comparison of a personalized sequencing assay and digital PCR for circulating tumor DNA based Molecular Residual Disease detection in a triple-negative triple negative breast cancer in the cTRAK-TN trial. *Cancer Res.* **83**, PD5−03 (2023).
4. Elliott, M. J. *et al.* Abstract PS-01-16: Circulating Tumour DNA (ctDNA) Detection and Dynamics in Patients with Early Breast Cancer (EBC): Results of the Neoadjuvant TRACER cohort. *Cancer Res.* **83**, PS−01 (2023).
5. García-Murillas, I. *et al.* Ultra-sensitive ctDNA mutation tracking to identify molecular residual disease and predict relapse in patients with early breast cancer. *J. Clin. Oncol.* **42**, 1010−1010 (2024).
6. The National Genomics Research Library v5.1, Genomics England. doi:10.6084/m9.figshare.4530893/7. 2020.