Longitudinal Neoadjuvant and Post-operative Evaluation of Circulating Tumor DNA in Early Breast Cancer Using a Tumor-Informed Assay: Updated Analysis of the TRACER Cohort

Mitchell J. Elliott[1], Jesús Fuentes Antrás[1], Philippe Echelard[2], Aaron Dou[1], Zachary Veitch[1], Eitan Amir[1], Michelle B. Nadler[1], Nicholas Meti[3], Nancy Gregorio[1], Elizabeth Shah[1], Celeste Yu[1], Nathan Campbell[4], Christodoulos Pipinikas[4], Karen Howarth[5], Lillian L. Siu[1], Philippe L. Bedard[1], Hal K. Berman[2], David W. Cescon[1]

1. Division of Medical Oncology & Hematology, Department of Medicine, Princess Margaret Cancer Centre and University of Toronto, Toronto, ON 2. Department of Pathology and Laboratory Medicine, University Health Network, Toronto, ON 3. Gerald Bronfman Department of Oncology, St. Mary's Hospital Center, McGill University, Montréal, QC 4. NeoGenomics, Babraham Research Campus, Cambridge, UK, Research Triangle Park, NC, USA 5. Bradfield Centre, Cambridge Science Park, Cambridge, United Kingdom

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Corresponding author: dave.cescon@uhn.ca

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INTRODUCTION

- Breast cancer is a leading cause of cancer-related death in women worldwide with disease often diagnosed in the "early" or localized setting.[1]
- Many patients diagnosed with early breast cancer (EBC) receive neoadjuvant systemic therapy (NAT), usually in the form of multi-agent chemotherapy, delivered in a risk-adaptive strategy based on routine clinical factors.[2]
- Despite the routine use of radiographic and pathologic assessment, a full understanding of an individual patient's recurrence risk is not fully understood nor characterized.[3–5]
- The assessment of ctDNA and associated therapy-related dynamic changes in EBC has been shown to be prognostic.[6] However, the prognostic utility of next-generation highly sensitive assays is currently unknown.
- RaDaR® (NeoGenomics Laboratories) is a personalized, tumor-informed assay, capable of detecting ctDNA with high sensitivity and specificity via deep sequencing of up to 48 tumor-specific variants, identified by tumor-only whole exome sequencing.
- ctDNA detection with the RaDaR assay after the completion of curative intent therapy is associated with an imminent risk of disease recurrence in high risk HR-positive (HR+) and triple negative (TNBC) EBC.[7,8]
- Early data illustrating the performance of the RaDaR assay in the neoadjuvant setting combined with adjuvant surveillance was previously evaluated.[9] Here we present an updated analysis of the TRACER cohort.

METHODS

- Patients with EBC of all receptor subtypes receiving standard of care NAT at the Princess Margaret Cancer Centre were enrolled in a prospective cohort between October 2016 and February 2022 (NCT03702309).
- Plasma samples (3 x Streck tubes) were collected at baseline, during treatment, perioperatively, and during adjuvant follow-up under the IRB-approved study protocol (**Figure 1**).
- All participants enrolled in TRACER up until August 2021 with available tissue were included in this cohort.
- Somatic variants were identified through whole exome sequencing of available archival formalin-fixed, paraffin embedded tissue from a diagnostic biopsy or surgical pathology (residual disease) to design personalized RaDaR assays (**Figure 2**).
- The buffy coat fraction was sequenced to identify confounding signals derived from clonal hematopoiesis of indeterminate potential and germline mutations (**Figure 2**).
- Personalized ctDNA RaDaR assays was performed on all available plasma samples by NeoGenomics. ctDNA positivity was defined by NeoGenomics based on predefined thresholds and metrics (**Figure 2**).
- Clinical and pathologic characteristics, treatment, and recurrence outcomes were collected.
- Recurrence outcomes were last updated on September 1, 2023.

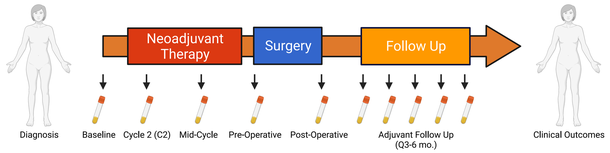


Figure 1. TRACER Study Design. Illustration of targeted timepoints for plasma collection with reference to a participant's clinical timeline.

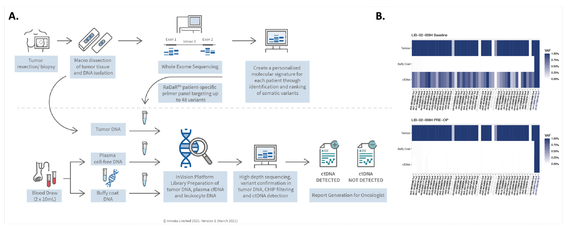
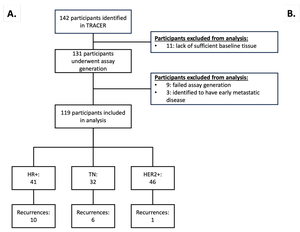


Figure 2. RaDaR Assay Preparation. (A) WES is performed on tumour tissue and used to generate a personalized RaDaR panel for each patient. Selected clinical time points are processed for ctDNA detection with the tumor variant specific MRD panel (RaDaR). **(B)** Variants detected in heatmaps generated for LIB-02-0084 (HER2+) at baseline and pre operative timepoints.

RESULTS

- 142 participants were identified from TRACER with 119 included in this primary analysis (**Figure 3A and B**).
- 681 plasma samples were analyzed from all collected timepoints.
- Median clinical follow-up from diagnosis was 3.9 years (range: 0.6-6.6 years).
- As of the data cut-off date, 17 recurrences (1 local, 16 distant) have occurred (HR+ n=10, TNBC n=6, HER2+ n=1).
- Baseline samples were available for 114 patients. Detection rate in all participants was 77% (88/114). Baseline detection varied by receptor subtype: HR+ 70% (28/40), TNBC 90% (26/29), and HER2+ 76% (34/45).
- Median estimated variant allele frequency (eVAF) in baseline samples with detectable ctDNA was 0.0823% (range: 2.90×10^{-5} to 7.5%).



B.

	All Patients (n=119)	HR-Positive (n=41)	HER2-Positive (n=46)	Triple Negative (n=32)
Age (Years)	49 [24-79]	48 [32-77]	49.5 [33-79]	50.0 [24-70]
Sex				
Female	118 (99.2%)	40 (97.6%)	46 (100%)	32 (100%)
Male	1 (0.8%)	1 (2.4%)	0 (0%)	0 (0%)
Menopausal Status				
Premenopausal	76 (63.9%)	29 (71.4%)	27 (58.7%)	20 (62.5%)
Postmenopausal	42 (35.3%)	11* (26.2%)	19 (41.3%)	12 (37.5%)
Clinical Features				
Tumour Size				
T1/2	79 (66.4%)	20 (48.8%)	34 (73.9%)	25 (78.1%)
T3/4	40 (33.6%)	21 (51.2%)	12 (26.1%)	7 (21.9%)
Nodal Status				
N-negative	46 (38.7%)	16 (39.0%)	11 (23.9%)	19 (59.4%)
N-positive	73 (61.3%)	25 (61.0%)	35 (76.1%)	13 (40.6%)
Neoadjuvant Treatment				
Anthracycline + Taxane	102 (85.7%)	38 (92.7%)	36 (78.3%)	28 (87.5%)
Taxane	14 (11.8%)	0 (0.0%)	10 (21.7%)	4 (12.5%)
Endocrine Therapy	3 (2.5%)	3 (7.1%)	0 (0%)	0 (0%)
Recurrences	17 (14.3%)	10 (24.4%)	1 (2.2%)	6 (19.4%)

Figure 3. Participant Selection and Baseline Clinical Variables. (A) Representative CONSORT diagram. **(B)** Baseline clinical variables in participants.

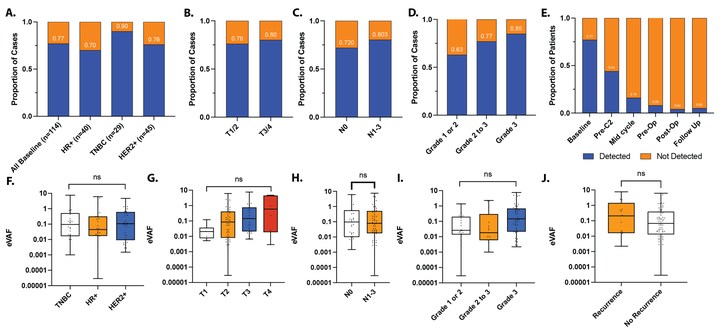
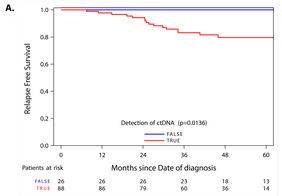


Figure 4. Baseline Clinical Features and ctDNA Detection. (A) Baseline ctDNA detection rate in all participants and by receptor subtype. Association between baseline ctDNA detection and clinical features **(B)** T-stage (p=0.599) **(C)** Nodal status (p=0.313) **(D)** Grade (p=0.0498). **(E)** Rate of ctDNA detection by timepoint. eVAF association with clinical features **(F)** Receptor subtype **(G)** T-stage **(H)** Nodal status **(I)** Grade and **(J)** Recurrence status.



B.

Patient ID	Receptor Subtype	Recurrence Type	Site of Recurrence	Lead time (days)	Time from last negative sample (days)
LIB-02-0020	HR+	Distant	Bone, Lung	862	1303
LIB-02-0067	HR+	Distant	Bone	13	289
LIB-02-0076	HR+	Distant	Liver, Lung	78	–
LIB-02-0088	HR+	Distant	Liver, Soft Tissue	28	475
LIB-02-0089	HR+	Distant	Lung, Bone	–	–
LIB-02-0116	HR+	Distant	Liver, Bone	748	–
LIB-02-0122	HR+	Distant	Lung, Bone, Lymph Node	–	–
LIB-02-0127	HR+†	Distant	Lymph Node	595	672*
LIB-02-0155	HR+	Distant	Liver, Bone	152	–
PUB-02-0011	HR+	Distant	Bone	–	–
LIB-02-0053	HER2+	Local	Breast	Not Detected	4
LIB-02-0085	TNBC	Distant	Lung, Lymph Node, Bone	–	–
LIB-02-0093	TNBC	Distant	Lung, Liver, Bone	–	–
LIB-02-0113	TNBC	Distant	Lung, Lymph Node	At Recurrence*	–
LIB-02-0121	TNBC	Distant	Lymph Node, Soft tissue	–	328
LIB-02-0126	TNBC	Distant	Lung	–	72
LIB-02-0151	TNBC	Distant	Liver, Lymph Node	–	–

Figure 5. Participant Outcomes. (A) Baseline ctDNA detection and association with Relapse-Free Survival (RFS; p=0.0136). **(B)** Participants with clinical recurrence. **Median lead time, 152 days (range, 13-862 days)**. Lead time was calculated retroactively from the date of clinical recurrence to the first positive test after definitive therapy (postoperative or in follow-up, whichever is earlier). – Lead time not able to be calculated. *Pre-operative timepoints positive. *Pre-operative timepoint. †Pathology demonstrating mixed HR-low (ER: 5%, PR: 0%) and TNBC with a TNBC recurrence.

RESULTS

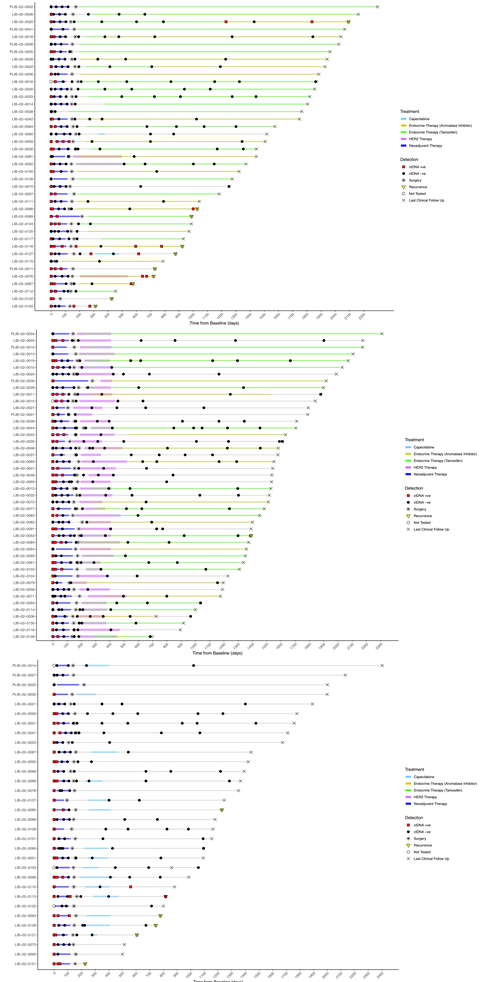


Figure 6. Representative Swimmer Plots. Swimmer plots demonstrating RaDaR results and clinical information for individual participants. **HR+:** top plot. **HER2+:** middle plot. **TNBC:** bottom plot.

RESULTS

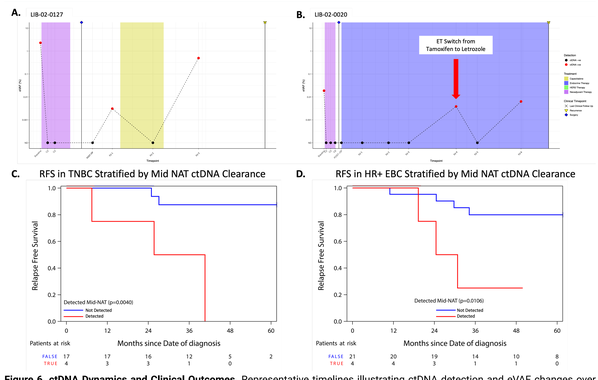


Figure 6. ctDNA Dynamics and Clinical Outcomes. Representative timelines illustrating ctDNA detection and eVAF changes over time with dynamic, treatment-related changes in the neoadjuvant and adjuvant setting in **(A)** LIB-02-0127 (HR-low) and **(B)** LIB-02-0020 (HR-positive). RFS in patients with **(C)** TNBC (p=0.0040) and **(D)** HR+ EBC (p=0.0106) stratified by mid-NAT ctDNA detection.

CONCLUSIONS

- RaDaR is a sensitive, tumor-informed assay, which detects ctDNA at baseline in most patients prior to NAT.
- The rate of baseline ctDNA detection varied by receptor subtype and was significantly associated with clinical grade but not other baseline clinical variables.
- ctDNA detection at baseline was prognostic. All participants experiencing disease recurrence had detectable ctDNA at baseline.
- Persistent ctDNA detection measured midway through NAT was prognostic of disease relapse in HR+ and TNBC.
- Clearance of ctDNA was observed with initiation or change in adjuvant systemic therapy, but relapses occurred if this was not durable.
- ctDNA can be detected using RaDaR with long lead times prior to clinical recurrence. The clinical utility of monitoring and interventional strategies require prospective evaluation.

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