Tumor-Agnostic Detection and Monitoring of Circulating Tumor DNA in HER2-positive Early Breast Cancer

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INTRODUCTION

- Detection of circulating tumor DNA (ctDNA) after definitive treatment, referred to as molecular or minimal residual disease (MRD), is associated with disease recurrence in early-stage breast cancer (EBC).[1-5]
- Differential methylation patterns enable tumor-agnostic ctDNA detection by distinguishing tumor-derived cell-free DNA (cfDNA) from background cfDNA.
- Tumor-agnostic assays do not require prior sequencing of tumor tissue, making them more accessible in settings where archival tissue is limited or unavailable.
- Data evaluating MRD using a tumor-agnostic approach in HER2-positive EBC remain limited.
- Methylation features can classify tumors based on their primary site.[6] The ability to predict receptor subtype using a methylation-based approach is of interest in EBC.

METHODS

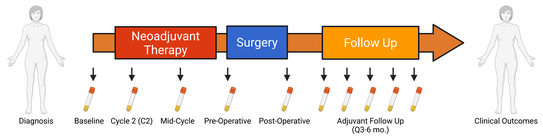


Figure 1. TRACER Study Design. Illustration of targeted timepoints for plasma collection with reference to a participant's clinical timeline. TRACER: *ctDNA evaluation in early breast cancer study* (NCT03702309)

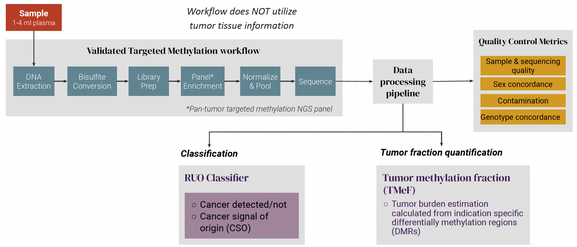


Figure 2. GRAIL Assay Pipeline and Classifier. Representative workflow of the GRAIL blood-only Cancer Research Solution (Research Use Only, RUO). Note: detection of cancer signal is independent of the Tumor Fraction (TMeF).

RESULTS

- 276 samples from 34 participants were retrospectively analyzed
  - Median time points per patient: 8 (range: 4-13)
- Median clinical follow-up was 4.8 years (range: 3.0-7.0 years)
- 270/276 (98%) passed quality control metrics (Failures: 1 genomic DNA contamination, 3 genotype mismatch, 2 both)
- **ctDNA was detected at baseline in:**
  - All participants [Stage I-III]: 75% (24/32)
  - HR+/HER2+: 65% (13/20)
  - HR-/HER2+: 92% (11/12)
- Baseline median TMeF was 0.067% (range: 0.00036-3.0%)
- **ctDNA was detected in:**
  - 10/94 of on-treatment and pre-operative timepoints **(Figure 3)**
  - 0/144 of post-operative and follow up timepoints (2 recurrences: 1 local, 1 isolated CNS; **Figure 4**)

Variable	N = 34
Age at Diagnosis	
30	6 (18%)
40	7 (21%)
50	10 (29%)
60	8 (24%)
70	3 (8.8%)
Sex	
Female	34 (100%)
Menopause Status	
Post	17 (50%)
Pre	17 (50%)
Clinical N Stage (AJCC 8th, prognostic)	
IA	1 (2.9%)
IIA	7 (21%)
IIB	16 (47%)
IIB / IIA (bilateral)	1 (2.9%)
IIIA	5 (15%)
IIIB	3 (8.8%)
IIIC	1 (2.9%)
Histology	
IDC	33 (97%)
Invasive carcinoma, apocrine features	1 (2.9%)
ER-status	
Negative	13 (38%)
Positive	20 (59%)
Positive/Negative (P/L)	1 (2.9%)
PR-status	
Negative	16 (47%)
Positive	16 (47%)
Positive/Negative (P/L)	1 (2.9%)
Unknown	1 (2.9%)
HER2-status	
Positive	33 (97%)
Positive/Positive	1 (2.9%)
HER2 IHC Score	
2+	3 (8.8%)
3+	30 (88%)
3+/3+	1 (2.9%)
Grade (Diagnostic Biopsy)	
2	8 (24%)
2 to 3	9 (26%)
3	17 (50%)
Residual Cancer Burden	
RCB-0	19 (56%)
RCB-1	9 (26%)
RCB-2	5 (15%)
RCB-3	1 (2.9%)
Neoadjuvant Regimen	
ddAC-T	1 (2.9%)
ddAC-TH	9 (26%)
ddAC-THP	7 (21%)
FEC-DH	9 (26%)
FEC-DHP	1 (2.9%)
TCH	3 (8.8%)
TCHP	1 (2.9%)
TH	3 (8.8%)

Table 1. Participant Characteristics.

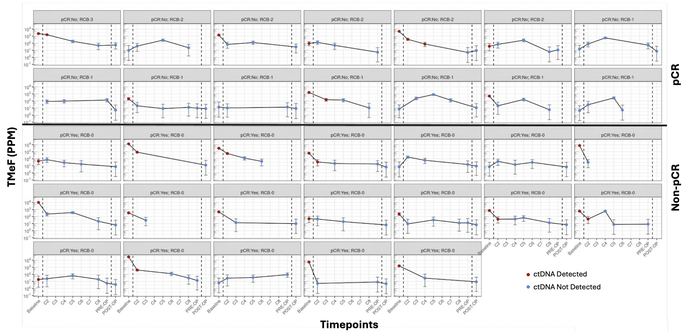


Figure 3. Tumor Methylation Fraction Dynamics on Neoadjuvant Chemotherapy. Individual participant timelines separated by non-pathologic complete response (non-pCR; top) and pCR (bottom). Longitudinal detection of ctDNA using TMeF and cancer signal classifier. TMeF plotted with point estimate and 95% confidence intervals. Dashed mark: the end of the neoadjuvant treatment window. Individual pCR status and residual cancer burden (RCB) scores are outlined.

RESULTS

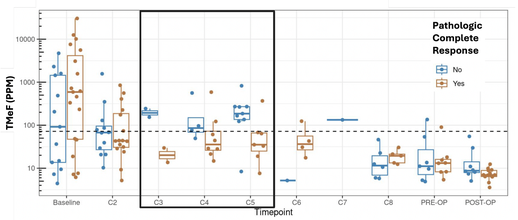


Figure 4. On-Treatment ctDNA Dynamics and Association with Pathologic Complete Response. Participants with residual disease at the time of surgery had a trend towards higher TMeF levels between C3-C5 of neoadjuvant chemotherapy. Dashed line: exploratory TMeF cutoff to maximize separation between pCR and non-pCR.

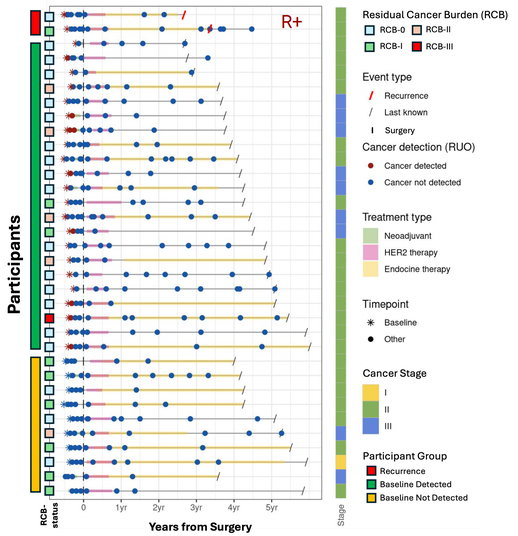
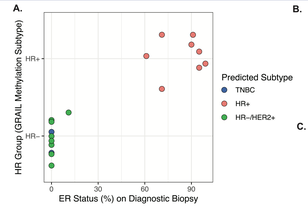


Figure 5. Representative Swimmer Plot. Longitudinal representation of ctDNA detection with respect to a participant's clinical timeline. ctDNA was not detected in post-operative samples before recurrence (R+) in 2 participants: local recurrence (2 mm; last test: 4 days prior) and an isolated CNS recurrence (last test: 193 days prior). No ctDNA was detected postoperatively or during adjuvant follow-up in the 32 recurrence-free participants (100% specificity).

RESULTS



B.

Pathologic Subtype (Biopsy)	N TMeF >100PPM (0.01% at baseline	Predicted Subtype (Methylation)		
		TNBC	HR+	HR-/HER2+
HR+/HER2+	10	0	8/10 (80%)	2/10 (20%)
HR-/HER2+	9	2/9 (22%)	0	7/9 (78%)

C.

Patient ID	ER status	HER2 status	CEP Ratio	Methylation Subtype
35	Negative	2+	3.49	TNBC
57	Negative	2+	2.2	TNBC

Figure 6. Methylation-based Molecular Subtyping Classification. (A) Distribution of methylation-based molecular subtyping by ER-status (%) on the diagnostic biopsy. Non-cancer classified when TMeF is low. (B) Distribution of methylation-based molecular subtype by pathologic subtype on the diagnostic biopsy. (C) 2/2 participants with HER2 IHC 2+ (FISH+) classified as TNBC by methylation-based subtyping. HR: hormone receptor.

CONCLUSIONS

- Baseline ctDNA detection was 75% overall and 92% in participants with HR−/HER2+ EBC
- This tumor-agnostic assay demonstrates high technical success, sensitivity and specificity, even at a low tumor methylation fraction
- Methylation-based molecular subtypes are associated with HR status in HER2+ EBC using this classifier
- Additional analyses in larger cohorts are warranted to further evaluate the validity and utility of ctDNA monitoring and methylation-based subtyping

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Société canadienne du cancer

REFERENCES

1. Cescon, D. W., Bratman, S. V., Chan, S. M. & Siu, L. L. Circulating tumor DNA and liquid biopsy in oncology. Nature Cancer (2020).
2. Coombes, R. C. et al. Personalized Detection of Circulating Tumor DNA Antedates Breast Cancer Metastatic Recurrence. Clin. Cancer Res. 25, 4255–4263 (2019).
3. Magbanua, M. J. M. et al. Circulating tumor DNA in neoadjuvant-treated breast cancer reflects response and survival. Ann. Oncol. 32, 229–239 (2021).
4. Parsons, H. A. et al. Sensitive Detection of Minimal Residual Disease in Patients Treated for Early-Stage Breast Cancer. Clin. Cancer Res. 26, 2556–2564 (2020).
5. Lipsyc-Sharf, Marla, Elza De Bruin, Katheryn Santos, Robert McEwen, Daniel Stetson, Ashka Patel, Gregory J. Kirkner, et al. 2022. "Circulating Tumor DNA (ctDNA) and Late Recurrence in High-Risk, Hormone Receptor–positive, HER2-Negative Breast Cancer (CHRR)." *Journal of Clinical Orthodontics: JCO* 40 (16_suppl): 103–103.
6. Nance, T. *et al.* Abstract 7566: Identification of cancer subtypes with a ctDNA-based targeted methylation assay. *Cancer Res.* **84**, 7566–7566 (2024).

Conflicts of Interest (D.W. Cescon): Dr. Cescon reports financial and non-financial support from GRAIL during the conduct of the study. He also reports consultancy and advisory relationships with AstraZeneca, Exact Sciences, Daiichi Sankyo, GenomeRx, Gilead, GlaxoSmithKline, NeoGenomics, Lilly, Merck, Novartis, Pfizer, Roche and SAGA Dx; research support (to the institution) from AstraZeneca, Guardant Health, Gilead, GlaxoSmithKline, NeoGenomics, Knight, Merck, Pfizer, ProteinQure, and Roche outside the submitted work.