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Ultra-sensitive ctDNA detection and monitoring in early breast cancer using PhasED-Seq

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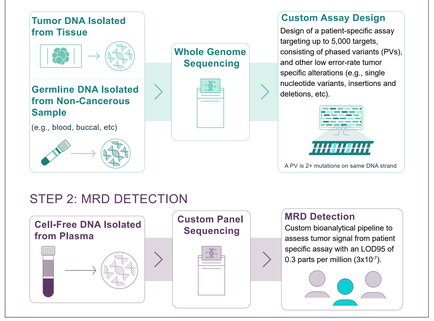
Background

- Detection of circulating tumor DNA (ctDNA) after treatment in early-stage breast cancer is associated with poor outcomes.
- 1st generation ctDNA methods are often hampered by suboptimal sensitivity, limiting performance in difficult scenarios including early-stage disease, hormone receptor-positive disease, and post-operative detection.
- We assessed the clinical validity of an ultra-sensitive ctDNA minimal residual disease (ctDNA-MRD) assay at key landmarks across various early-stage breast cancer subtypes.

Methods

- Here, we present the analysis of the first 50 patients from this ongoing study of ctDNA-MRD in early breast cancer patients.
- These patients were from the MSK-LINC study, which prospectively collected blood samples from patients with early breast cancer throughout their clinical care at MSK (IRB protocols 12-245, 06-107).
- Foresight CLARITY™, an ultra-sensitive tumor-informed MRD assay built on Phased Variant Enrichment and Detection Sequencing, was used to assess for MRD (**Figure 1**).

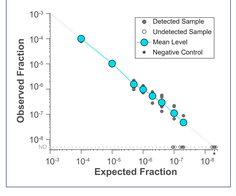
Figure 1. Overview of MRD Testing Process



- Whole genome sequencing of primary tumor and white blood cell samples was utilized to identify tumor-derived variants.
- Personalized MRD assays were designed and applied to plasma to detect ctDNA-MRD and reported as tumor fraction.

- The limit of detection (LOD95) of the MRD assay is 0.3 parts per million (ppm; **Figure 2**).
  - 3 limiting dilution series of cell-free DNA (cfDNA) from non-small cell lung cancer samples were performed, with NSCLC cfDNA diluted into cancer-free donor background cfDNA.
  - Dilution series were performed in triplicate from expected tumor fraction of 1 in 10,000 to 1 in 20,000,000.
  - LOD95 was determined by Probit modeling to be $3x10^{-7}$.
  - Background signal rate was 1 in 35 million.
  - Additional negative samples were assessed for specificity (100%).

Figure 2. LOD of MRD Assay



Results

- In this study, 390 samples from 50 patients (**Table 1**) were analyzed.
  - 36% of pre-treatment and 68% of post-treatment samples with detectable ctDNA had ctDNA levels <0.01% (<100 ppm).
- Levels of ctDNA were assessed prior to treatment (**Figure 3**).
  - 72% of patients had detectable ctDNA pre-treatment.
  - Pre-treatment ctDNA detection was associated with tumor stage, tumor status, nodal status, and grade (**Figure 3B-3E**).
  - Tumor subtype was not statistically associated with ctDNA detection (**Figure 3A**).
- Levels of ctDNA were assessed at landmarks related to neo-adjuvant chemotherapy (NACT) for patients who received NACT (**Figure 4A**).
  - Patients who achieved pathological complete response (pCR) all cleared ctDNA prior to surgery.
  - All samples obtained post-NACT with detectable ctDNA had tumor fractions $<10^{-4}$ (the approximate sensitivity limit for 1st generation ctDNA-MRD assays).
- Levels of ctDNA were assessed at landmarks related to surgery (**Figure 4B**).
  - Many samples at pre- and post-operative time points, as well as during follow-up, had ctDNA detectable with tumor fractions $<10^{-4}$.
  - Patients who did not experience disease relapse had lower levels of ctDNA at post-operative and follow-up time points than those who experienced disease relapse.
- Levels of ctDNA change during therapy, with clearance of ctDNA-MRD observed at various landmark time points throughout therapy, including after NACT, surgery, adjuvant chemotherapy, and endocrine therapy (**Figure 5**).
- **Figure 6** shows the clinical history and ctDNA detection for all samples for all patients considered in this study.
  - All patients with disease progression had ctDNA detected at or prior to the time of relapse (n=7/7; 100% sensitivity).
  - All patients with durable remission had persistently undetectable ctDNA during follow-up, generally after clearance of prior detectable ctDNA from NACT, surgery, or adjuvant therapy.

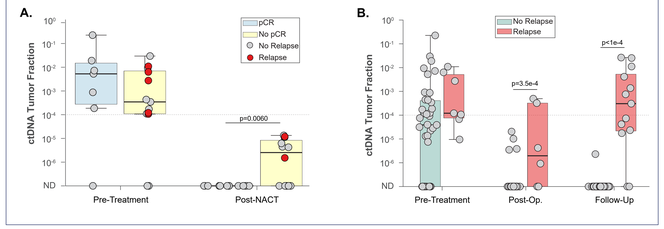
Table 1. Demographics (N=50)

Age, median (IQR)		51 (46, 60)
Subtype	HR+/HER2-	30 (60%)
	TNBC	8 (16%)
	HER2+	12 (24%)
Histology	Ductal (NST)	43 (86%)
	Lobular	6 (12%)
	Others	1 (2%)
Grade	I	1 (2%)
	II	8 (16%)
	III	41 (82%)
Stage	I	11 (22%)
	II	28 (56%)
	III	11 (22%)
Tumor Size*	T1	14 (28%)
	T2	24 (48%)
	T3-4	11 (22%)
Node Positive		26 (52%)
NACT		22 (44%)
Adjuvant Chemotherapy		24 (48%)

*N=49 for tumor size, one T0 excluded. HER2, human epidermal growth factor receptor 2; HR, hormone receptor; IQR, interquartile range; NACT, neo-adjuvant chemotherapy; NST, no special type; TNBC, triple negative breast cancer

Figure 4. ctDNA Levels at Landmark Time Points in ctDNA+ Patients

Dashed line indicates the approximate LOD for 1st generation assays. (A) Treatment landmarks related to neo-adjuvant chemotherapy (NACT), with patients grouped by pathological complete response (pCR) status. Patients who eventually relapse are indicated by red circles. (B) Treatment landmarks related to surgery, with patients grouped by eventual relapse status.



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References: 1. Kurtz, D.M., et al. Nat Biotechnol 39, 1537–1547 (2021).

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Figure 3. Distribution of Pre-Treatment ctDNA Levels by Disease Characteristics

The correlation between tumor fraction (i.e. ctDNA level) prior to treatment and clinical characteristics was assessed. Black dashed line indicates the approximate LOD for 1st generation assays. Grey dash line indicates not detected (ND).

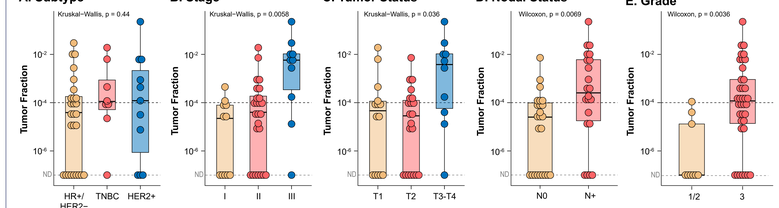


Figure 5. Individual Patient Vignettes

Vignettes highlighting individual patients who (A) did not clear ctDNA after treatment or who had undetectable ctDNA after (B) neo-adjuvant chemotherapy, (C) surgery, (D) adjuvant chemotherapy, or (E) endocrine therapy. ND indicates that ctDNA is not detectable. Demographic information indicated in inset text for each vignette.

Figure 6. Swimmer Plot of ctDNA Detection

Each line indicates an individual patient, grouped by breast cancer subtype.

Results (Continued)

Figure 7. Disease-Free Survival (DFS) Stratified by Pre-treatment ctDNA Detection

No recurrence was observed in patients who had undetectable ctDNA at baseline.

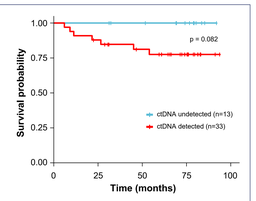


Figure 8. DFS Stratified by MRD Status Post-Surgery

In landmark analysis, 8/49 (16%) of patients were MRD+ post-surgery (first sample after surgery, <250 days).

MRD positivity after surgery was associated with DFS (hazard ratio 14.0, 95% CI 1.2-158).

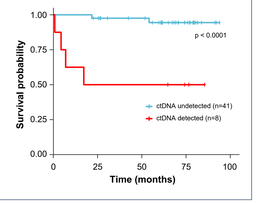
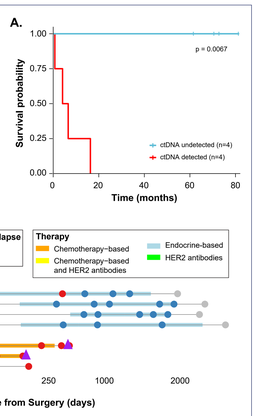


Figure 9. DFS Stratified by MRD Clearance after Adjuvant Therapy

(A) In the 8 post-operative MRD+ patients, adjuvant therapy cleared MRD in 4 (50%); none experienced relapse. One patient relapsed before adjuvant therapy. The remaining 3 patients failed to clear MRD with adjuvant therapy, all of whom relapsed.

(B) Swimmer plot showing all 8 patients. The top 4 lines indicate patients who cleared and did not relapse. The bottom 4 lines indicate patients who did not clear and did relapse.



Conclusions

- Foresight CLARITY™ is a highly sensitive and specific assay for MRD detection in early breast cancer, with a technical LOD95 of 0.3 ppm.
  - This enabled disease detection of samples below 0.01% tumor fraction, including 36% and 68% of pre-treatment and post-treatment samples, respectively.
- Clearance of ctDNA-MRD can be observed at each phase of therapy and is associated with clinical outcomes.
- Post-surgical ctDNA-MRD positivity was associated with recurrence and subsequent clearance of ctDNA-MRD by adjuvant therapy further predicted DFS.
- Trials to assess the utility of this ctDNA-MRD assay in identification of high-risk patients for escalated therapy are warranted.