Circulating tumor DNA clearance by neoadjuvant chemotherapy or breast surgery detected using an ultrasensitive ctDNA MRD assay in early breast cancer

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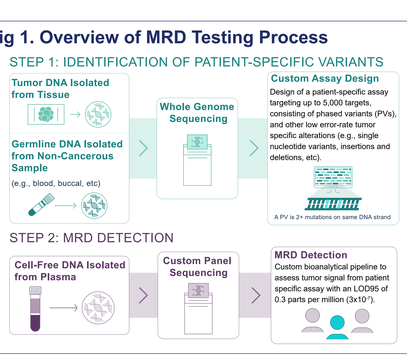
Background

- 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.
- Here we present the clinical validity of an ultra-sensitive ctDNA minimal residual disease (ctDNA-MRD) assay at key landmarks across early-stage breast cancer subtypes, using the first 81 patients from an ongoing study.

Methods

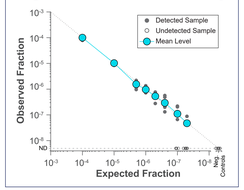
- Patients were enrolled from the MSK-LINC study, which prospectively collected blood samples from patients with early breast cancer throughout their clinical care at MSK (IRB protocols 12-245, 06-107).
- Foresight CLARITY™, an ultra-sensitive tumor-informed MRD assay built on Phased Variant Enrichment and Detection Sequencing,[1] was used to assess for MRD (**Fig. 1**).

Fig 1. Overview of MRD Testing Process



- The limit of detection (LOD95) of the MRD assay is 0.3 parts per million (ppm; **Fig. 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.
  - Background signal rate was 1 in 35 million.
  - Negative samples were assessed for specificity (100%).

Fig 2. LOD of MRD Assay



Results

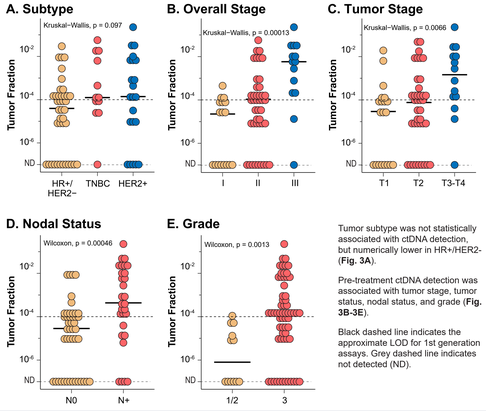
- In this study, 470 samples from 81 patients (**Table 1)** were analyzed.
  - 34% of pre-treatment and 68% of post-treatment samples with detectable ctDNA had ctDNA levels <10^{-4} (<0.01%), the approximate sensitivity limit for 1st generation ctDNA-MRD assays.
- Levels of ctDNA were assessed prior to treatment (**Fig. 3**).
  - 76% of patients had detectable ctDNA pre-treatment.
- Levels of ctDNA were assessed at landmarks related to neo-adjuvant chemotherapy (NACT) for patients who received NACT (**Fig. 4**).
  - 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}.
- Levels of ctDNA were assessed at landmarks related to surgery (**Fig. 5**).
  - 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.
  - Post-NACT, ctDNA clearance was observed in 87% of patients with detectable pre-treatment ctDNA.
- 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 (**Fig. 6-7**).
- **Fig. 7** shows the clinical history and ctDNA detection for all samples for all patients considered in this study.
  - All patients with disease progression who had end of treatment blood samples available had detectable ctDNA (n=9/9; 100% sensitivity).
  - All patients with durable remission had undetectable ctDNA at their last timepoint during follow-up. Further, all these patients except one had persistently undetectable ctDNA during the follow-up period, generally after clearance of ctDNA from NACT, surgery, or adjuvant therapy.

Table 1. Demographics (N=81)

Age, median (IQR)		51 (45, 60)
Subtype	**HR+/HER2-**	45 (56%)
	TNBC	12 (15%)
	HER2+	24 (30%)
Histology	**Ductal (NST)**	71 (88%)
	Lobular	8 (10%)
	Others	2 (2%)
Grade	**I**	1 (1%)
	II	12 (15%)
	III	67 (84%)
Stage	**I**	17 (21%)
	II	48 (59%)
	III	16 (20%)
Tumor Size*	**T1**	20 (25%)
	T2	44 (55%)
	T3-4	16 (20%)
Node Positive		33 (41%)
NACT		38 (47%)
Adjuvant Chemotherapy		38 (47%)

*N=80 for tumor size, one T0 excluded. 45% pathological complete reponse; 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

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



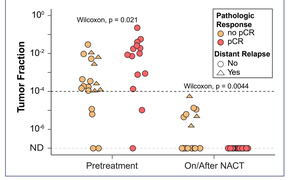
Tumor subtype was not statistically associated with ctDNA detection, but numerically lower in HR+/HER2- (**Fig. 3A**).

Pre-treatment ctDNA detection was associated with tumor stage, tumor status, nodal status, and grade (**Fig. 3B-3E**).

Black dashed line indicates the approximate LOD for 1st generation assays. Grey dashed line indicates not detected (ND).

Fig 4. ctDNA Levels at Baseline and End/After Neo-Adjuvant Chemotherapy

Black dashed line indicates the approximate LOD for 1st generation assays. Grey dashed line indicates not detected (ND). Wilxocon test was used to compare ctDNA levels for pCR and no pCR. End/Post-NACT samples collected ≤60 days prior to surgery.



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

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Fig 5. ctDNA Levels at Landmark Time Points Related to Surgery

(A) Upfront surgery cleared ctDNA in the majority of patients with detectable baseline ctDNA (84%).

(B) In patients who received NACT, surgery cleared ctDNA in patients without relapse, while all patients with detectable ctDNA post-surgery experienced a disease relapse. This highlights the origin of ctDNA detection as either from primary or micrometastatic disease.

Black dashed line indicates the approximate LOD for 1st generation assays. Grey dashed line indicates not detected (ND). End/Post-NACT samples collected ≤60 days prior to surgery.

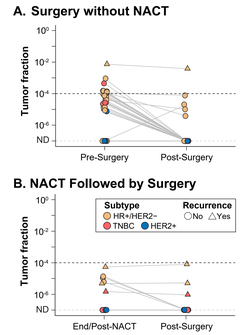


Fig 6. Individual Patient Vignettes

Vignettes highlighting individual patients who (A) had detectable ctDNA after both surgery and adjuvant therapy, and experienced relapse almost 3 years after surgery, or who had undetectable ctDNA after (B) surgery or (C) neo-adjuvant chemotherapy. Demographic information indicated in inset text for each vignette. The dashed line indicates the LOD for the ctDNA assay.

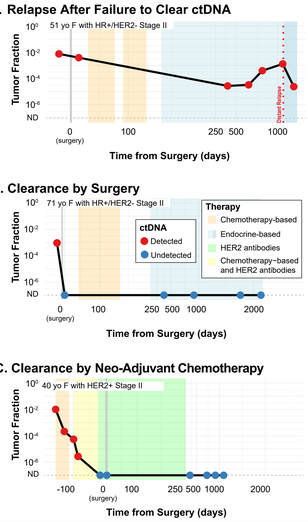


Fig 7. Swimmer Plot of ctDNA Detection

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

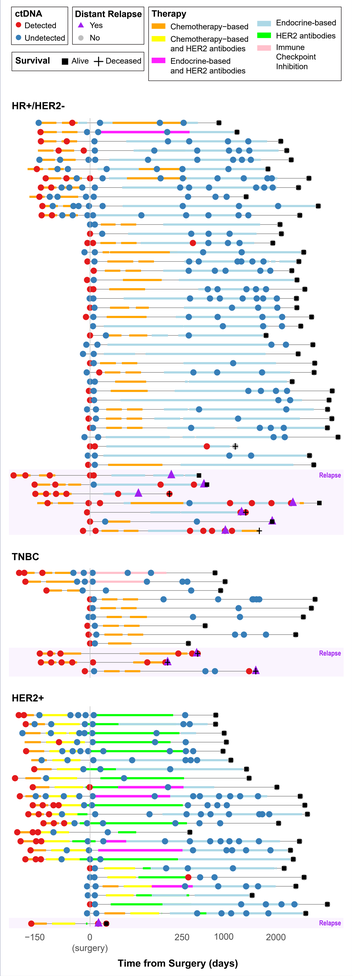


Fig 8. Disease-Free Survival (DFS)

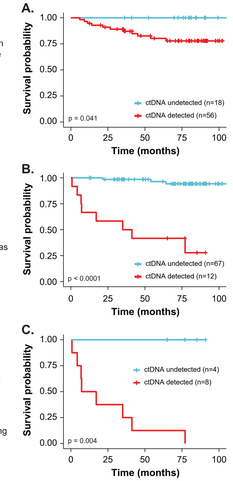
(A) **Stratified by Pre-Treatment ctDNA Detection**. No recurrence was observed in patients who had undetectable ctDNA at baseline.

(B) **Stratified by MRD Status Post-Surgery**. In landmark analysis, 12/79 (15%) of patients were MRD+ post-surgery (first sample after surgery, <250 days).

MRD positivity after surgery was associated with DFS (hazard ratio 21.4, 95% CI 5.6-80.9).

(C) **Stratified by MRD Clearance after Adjuvant Therapy**. In the 12 post-operative MRD+ patients, adjuvant therapy cleared MRD in 4 (33%); none experienced relapse.

One patient relapsed before adjuvant therapy. The remaining 7 patients failed to clear MRD with adjuvant therapy, all of whom relapsed or died.



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 10^{-4} tumor fraction, including 34% 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 improved clinical outcomes.
- Post-surgical ctDNA-MRD positivity was associated with recurrence, but subsequent clearance of ctDNA-MRD by adjuvant therapy further predicted long-term recurrence-free survival.
- Trials to assess the utility of this ctDNA-MRD assay in identification of high-risk patients for escalated therapy are warranted.

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