Early Detection of Residual Breast Cancer through a Robust, Scalable and Personalized Analysis of Circulating Tumour DNA (ctDNA) Antedates Overt Metastatic Recurrence

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Introduction

- The potential utility of circulating tumor DNA (ctDNA) to detect minimal residual disease (MRD) following surgery and/or adjuvant therapy and to monitor metastatic disease has been demonstrated in breast cancer.[1-4]
- Plasma ctDNA levels have been shown to correlate with changes in tumor burden, thereby providing an earlier measure of treatment response[5,6] and to discriminate patients with and without eventual clinical recurrence post-surgery.[2,3]
- We previously reported early detection of lung cancer recurrence using a personalized tumor-specific approach;[7,8] an improved version of this assay for patient-specific ctDNA detection was developed and made available for research use only (Signatera™ RUO).

Objectives

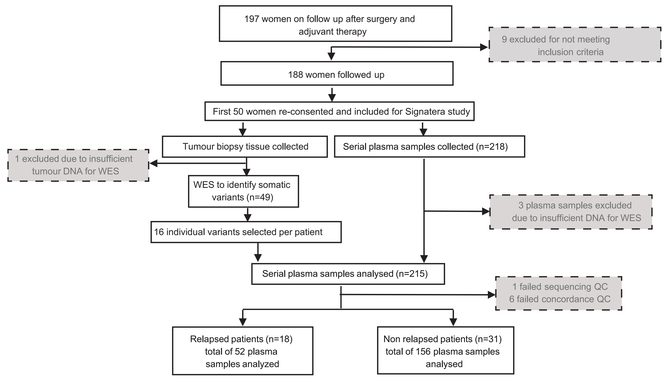
The aim of the study was to determine the 'lead interval' between detection of ctDNA in plasma and clinical detection of overt metastatic disease.

Methods

Clinical Protocol

- Patients diagnosed with non-metastatic breast cancer with high risk of reccurence were prospectively recruited starting in Dec 2013.
- Eligible patients were followed up with semi-annual blood sampling for ctDNA analysis, along with concomitant clinical examination, and biochemical measurements, including CA 15-3 (**Figure 1**).
  - All patients had completed adjuvant chemotherapy within 3 years of entering the study.

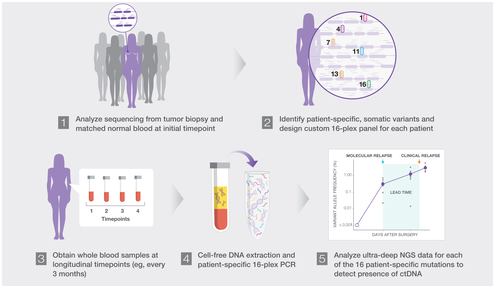
Figure 1. Schematic of Clinical Sample Collection



Molecular Protocol - ctDNA Analysis (Signatera™ RUO)

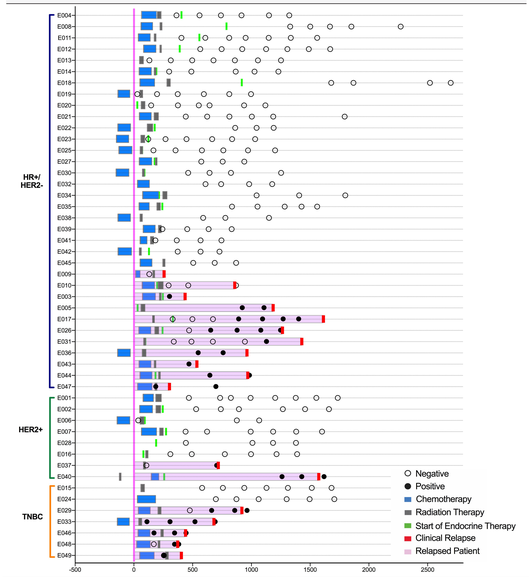
- The workflow of the molecular protocol is outlined in **Figure 2**.
- For the 49 women with breast cancer who were monitored in this study, exomic alterations were determined through paired-end sequencing of FFPE tumor-tissue specimens and matched normal DNA.
- Patient-specific somatic variants were identified by analyses of primary tumor and matched normal whole exome sequencing samples.
- Serial plasma samples were analyzed with the corresponding custom 16-plex assay panels using the Signatera™ RUO workflow in a blinded manner; a total of 208 samples were analyzed for ctDNA detection.
- Samples were considered ctDNA positive if at least two patient-specific targets were called and met the qualifying confidence score threshold.

Figure 2. Schematic of Molecular Protocol - ctDNA Analysis



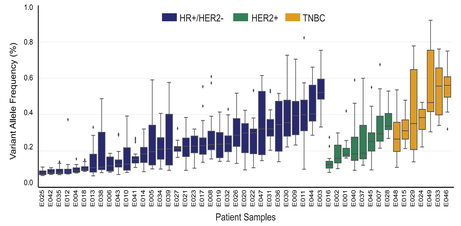
Results

Figure 3. Patient Summaries



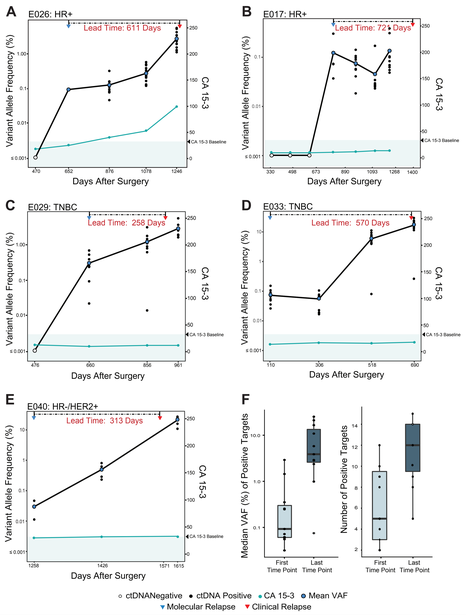
Personalized, 16-plex assays accurately detect ctDNA ahead of clinical relapse. The figure depicts a summary of each patient's (n=49) treatment regimen along with results of serial plasma samples (n=208) analyzed. Patients are categroized into three groups based on cancer subtype. Circles represent ctDNA status; solid black circles (ctDNA positive) indicate samples with at least two positive targets.

Figure 4. Tumor Variant Allele Frequency Distribution in Signatera Patient-Specific Assays



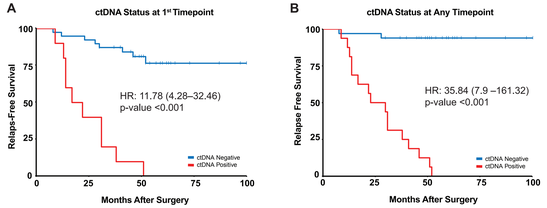
The VAF distribution of custom assay in each patient's primary tumour tissue

Figure 5. Personalized Profiling Detects Rising ctDNA Ahead of Clinical Relapse



A-E. Plasma levels of ctDNA across serial plasma time points for five breast cancer patients (one per panel). Mean VAFs are denoted by dark blue circle and solid lines represent the average VAF profile over time. The lead time is calculated as the time interval between clinical relapse (red triangle) and molecular relapse (blue triangle). CA 15-3 levels are graphed over time (teal circle) and the baseline levels are marked in light blue. **F.** Summary of percent variant allele frequency (VAF) and number of targets detected at molecular and clinical relapse for all ctDNA positive samples. Data are from 13 relapsed patients, excluding 3 patients with only one plasma time point.

Figure 6. Personalized ctDNA Detection in Serial Plasma Samples Predicts Recurrence-Free Survival



Personalized ctDNA detection in serial plasma samples predicts recurrence-free survival. **A.** Relapse-free survival according to the detection of ctDNA in the first post-surgical plasma sample. **B.** Relapse-free survival according to the detection of ctDNA in any follow-up plasma sample post-surgery. Data are from n=49 patients.

Table 1. Summary Table by Breast Cancer Subtype

Breast Cancer Subtype	Total Patients (n)	Relapsed Patients (n)	Relapses Detected n (%)	PPV (%)	NPV (%)	Median Lead Time (Days)
HR+/HER2-	34	11	9 (82)	100	92	301
HER2+	8	2	2 (100)	100	100	164
TNBC	7	5	5 (100)	100	100	258
Total	49	18	16 (89)	100	94	285

PPV, Positive Predicitive Value [True Positive/(True Positive+False Positive)]; NPV, Negative Predictive Value [True Negative/(False Negative+True Negative)]

Conclusions

- Plasma ctDNA was detected ahead of clinical or radiological relapse in 16/18 (89%) relapsed patients.
  - Of the two relapsed patients who were not detected in the study, one patient had a local recurrence while the other had recently completed chemotherapy, and neither of the relapses were confirmed by biopsy.
- Metastatic relapse was predicted with a lead time of up to 2 years (median=8.9 months; range: 0.5–24.0 months).
- None of the 31 non-relapsing patients were ctDNA-positive at any time point (across 156 plasma samples).
- Our results demonstrate the use of a scalable patient-specific ctDNA approach capable of identifying residual disease in breast cancer patients with a high degree of sensitivity ahead of clinical or radiological detection of metastatic recurrence.

References

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