Monitoring ctDNA dynamics in early breast cancer using a novel ultra-sensitive tumor-informed structural variant approach combining whole-genome sequencing and multiplex dPCR

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Introduction:

Preoperative chemotherapy in early breast cancer (EBC) increases the rate of breast preservation and provides prognostic information. In highly proliferative disease, a pathological complete response (pCR) to preoperative chemotherapy associated with beneficial outcome in terms of overall and event-free survival [1].

The analysis of circulating cell-free tumor DNA (ctDNA) has emerged as a powerful tool for the quantification and characterization of disease in plasma. Persistent ctDNA detection during neoadjuvant treatment of EBC indicates high-risk disease. Detection of cDNA post-resection of EBC molecular residual disease (MRD) indicates occult metastatic disease and impending disease relapse. For ctDNA to be integrated into EBC management, accessible and scalable diagnostic tools are required.

NeoCircle is a prospective clinical study for ctDNA monitoring of EBC patients eligible for neoadjuvant chemotherapy begun in December 2014 as part of the large population-based SCAN-B initiative (ClinicalTrials.gov identifier NCT02306096; [2]). The purpose of the study is to evaluate ctDNA as a tool for early response evaluation in different subtypes of breast cancer undergoing preoperative chemotherapy and for postoperative monitoring for MRD.

Here we report interim observations of the dynamics of ctDNA and surgical outcomes for the first 46 patients meeting quality control criteria. For ctDNA analysis, we employ the a highly sensitive, personalized tumor-informed approach predicated on analyses of structural variants (SVs) using a novel digital PCR (dPCR) SV technology (**Figure 1**). This method is an extension and improvement upon the approach we have previously reported [3].

Patients and Methods:

NeoCircle prospectively enrolls patients planned for preoperative chemotherapy with a curative intent for early or locally recurrent breast cancer. Clinical biopsy and fresh tumor tissue is collected through an ultrasound-guided core needle biopsy from the primary tumor, or a lymph node metastasis in case no primary tumor is localized in the breast. Blood samples (10 mL Streck Cell-Free BCT) for quantification of ctDNA are collected at the following time points: at baseline mammography, after the first and third three-weekly chemotherapy cycles, before and after definitive surgery, two weeks after surgery, and during follow-up at 6, 12, 18, 36, 48 and 60 months after inclusion in the study. A subset of patients also provided blood samples immediately after breast compression at baseline, as well as during and immediately after definitive surgery.

ctDNA analysis: The personalized tumor-informed approach predicated on analyses of structural variants (SVs) using a novel digital PCR (dPCR) SV technology. Tumor tissue biopsy DNA undergoes low-passage whole-genome sequencing, from which proprietary bioinformatics identifies a high-confidence clonal ”fingerprint” of the tumor’s SVs and SNVs, for which a personalized multiplex dPCR test is designed using a proprietary workflow (**Figure 1**). Cell-free DNA is isolated from 4-5 mL plasma (Streck BCT) generally within 2 days of phlebotomy. Multiplex dPCR containing a median of 8 fingerprint markers measures ctDNA down to a lower limit of detection of approximately 0.0001% MAF in this cohort. Interim results for the first 46 consecutive patients meeting QC requirements (10% tumor content and 10x sequencing depth) are reported here.

Pathological assessment: At definitive surgery, tissue for pathological response evaluation, assessment of surgical margins and clinical biomarker analysis was performed according to clinical routine. A pathological complete response (pCR) was defined as the complete disappearance of invasive cancer in the breast or axillary lymph nodes. In situ changes are allowed. Patients that have had a negative sentinel node biopsy at baseline, responded to preoperative chemotherapy, and did not have any additional axillary evaluation at time of definitive surgery, were considered to have a pCR if no invasive cancer cells were observed in the breast.

Treatment: Patients are treated according to national and regional treatment guidelines. Most patients receive sequential chemotherapy including an anthracycline based component (Epirubicin and Cyclophosphamide q3w x 3) and a taxane (docetaxel q3w x 3 or paclitaxel q1w x 9-12). HER2-directed antibodies are added as appropriate. Some patients receive an alternate sequence or just an anthracycline or taxane in case of, e.g., poor tolerability, hypersensitivity, or for practical reasons. Postoperative treatment includes endocrine treatment, radiotherapy, and zoledronic acid according to guidelines, and in selected cases capecitabine to patients with a poor response at conventional response evaluation.

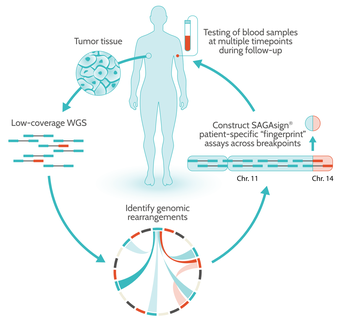


Figure 1 – Tumor-informed SV-dPCR method.

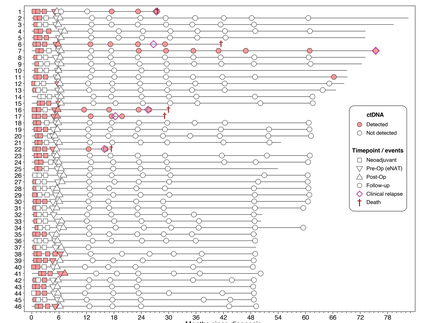
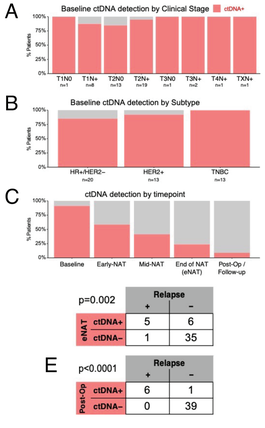


Figure 2 – First 46 patients, longitudinal monitoring.



p=0.002

		Relapse +	Relapse −
eNAT	ctDNA+	5	6
	ctDNA−	1	35

E p<0.0001

		Relapse +	Relapse −
Post-Op	ctDNA+	6	1
	ctDNA−	0	39

Figure 3 – Detection rates by (**A**) clinical stage at diagnosis, (**B**) breast cancer subtype, and by (**C**) timepoint. Contingency tables for (**D**) end-of-NAT ctDNA and (**E**) any post-op ctDNA detection *vs.* clinical relapse.

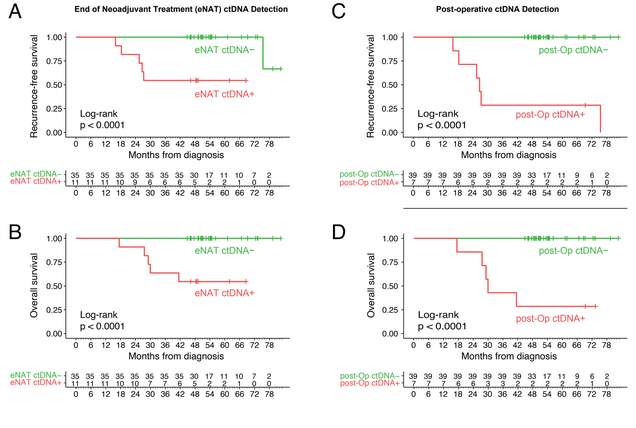


Figure 4 – Kaplan-Meier survival estimates for (**A**, **B**) end-of-NAT ctDNA detection or (**C**, **D**) post-op ctDNA detection for (**A**, **C**) recurrence-free survival and (**B**, **D**) overall survival.

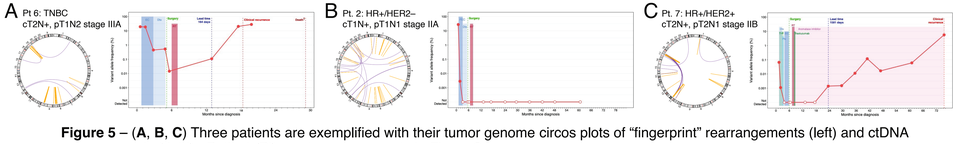


Figure 5 – (**A**, **B**, **C**) Three patients are exemplified with their tumor genome circos plots of “fingerprint” rearrangements (left) and ctDNA measurements (right). Patient (Pt) number corresponds to Figure 2.

Results:

170 patients with primary stage I-III breast cancer or a locoregional recurrence amenable for curative treatment have consented to the NeoCircle study between Dec 2014 and Mar 2019 (25.8% TNBC, 47.1% HR+/HER2- and 24.1% HER2+). Enrollment is closed; over 2000 blood samples have been collected and blood sampling is ongoing.

Interim results are presented for 567 plasma samples for the first 46 consecutive patients where minimum QC criteria were met for ctDNA analysis (**Figure 1** and **2**).

- cDNA was detected at one or more timepoints prior to surgery in 43/46 (93%) patients across all breast cancer subtypes and in 91% at baseline. Median variant allele frequency (VAF) prior to treatment was 0.14% (range 0.0002% - 27.6%) (**Figures 2** and **3**).
- cDNA levels remained detectable at the end of NAT in 24% patients (11/46); 5/11 (45%) cDNA positive patients experienced disease relapse versus 1/35 (3%) cDNA negative patients (p=0.002, Fisher's exact test) (**Figures 2** and **3**).
- At one or more post-operative timepoints, cDNA was detected in 6/6 (100%) patients who experienced clinical recurrence, with lead times up to 52 months (median 11.8 months, range 3.5 to 52 months) (**Figures 2**, **3**, **4**, and **5**).
- In 40 patients without presentation of clinical recurrence as of last follow-up, cDNA was undetectable across 285/287 (99.3%) plasma samples collected post-surgery (**Figures 2**, **3**, **4**, and **5**). In one case, positive ctDNA immediately post-surgery cleared with adjuvant therapy, and the other was censored close to the positive detection.
- End of neoadjuvant treatment (eNAT) detection of ctDNA associated with poor relapse-free survival (RFS) and overall survival (OS) compared to absence of ctDNA (log-rank p<0.0001) (**Figure 4A**, **4B**).
- Post-operative detection of cDNA associated with poor RFS and OS compared to absence of cDNA (log-rank p<0.0001) (**Figure 4C**, **4D**).

Preliminary conclusions:

- In this interim analysis of the prospective NeoCircle study of ctDNA monitoring in early breast cancer patients receiving neoadjuvant therapy (NAT), we analyzed plasma ctDNA using a novel tumor-informed dPCR assay tracking patient-specific structural variants.
- 93% of cases displayed informative levels of ctDNA before surgery (91.3% at baseline).
- ctDNA detection at end of NAT associated with high-risk for relapse and death.
- Post-operative detection of ctDNA was observed in 100% of patients with clinical recurrence and associated with poor RFS and OS and long lead-times.
- Our interim data demonstrate the feasibility of SVs as an MRD analyte and provide evidence for high levels of clinical sensitivity achievable with this approach in EBC.

References: 1. Cortazar P., et al. *CTRC-AACR San Antonio Breast Cancer Symposium*. 2012. San Antonio, Texas. 2. Saal LH., et al. *Genome Medicine*, 2015. Feb 2;7(1)20. 3. Olsson E.., et al. *EMBO Molecular Medicine*. 2015. Aug;7(8): 1034-47.

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