NeoCircle: circulating tumor DNA (ctDNA) dynamics during neoadjuvant chemotherapy predicts survival in primary breast cancer

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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 is 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 (NAT) of EBC indicates high-risk disease. Detection of ctDNA 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 the dynamics of ctDNA and outcomes for 136 NeoCircle patients meeting quality control criteria (**Figure 1A**). For ctDNA analysis, we employ a highly sensitive, personalized tumor-informed approach predicated on analyses of structural variants (SVs) using a novel digital PCR (dPCR) SV technology (**Figure 1B**). We have previously shown the high performance of ctDNA monitoring for breast cancer MRD using SVs [**3**].

Patients and Methods:

NeoCircle prospectively enrolled patients planned for preoperative chemotherapy with curative intent for early breast cancer (EBC). Clinical biopsy (FFPE) and fresh tumor tissue were 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 is predicated on analyses of structural variants (SVs) using a novel digital PCR (dPCR) SV technology [**4**]. Tumor tissue biopsy DNA undergoes low-passage whole-genome sequencing, from which proprietary bioinformatics identifies "fingerprint" of the tumor's SVs, for which a personalized multiplex dPCR test is designed using a proprietary workflow (**Figure 1B**). Cell-free DNA is isolated from 4-5 mL plasma (Streck BCT) generally within 2 days of phlebotomy. Multiplex dPCR containing up to 16 SV fingerprint markers with a limit of detection (LOD) at 95% certainty of 0.00052% tumor fraction (5 parts per million, PPM) with SVs detected below 1 part in 10 million (0.00001% or 0.1 PPM) in analytical validation studies, as well as 100% analytical specificity across 5,268 SV measurements of 217 control cfDNAs and 217 normal DNA samples [**4**].

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 guidelines. Most patients receive sequential chemotherapy including an anthracycline (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.

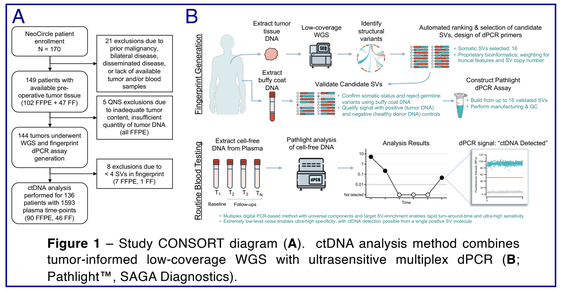

Figure 1 – Study CONSORT diagram (**A**). ctDNA analysis method combines tumor-informed low-coverage WGS with ultrasensitive multiplex dPCR (**B**; Pathlight™, SAGA Diagnostics).

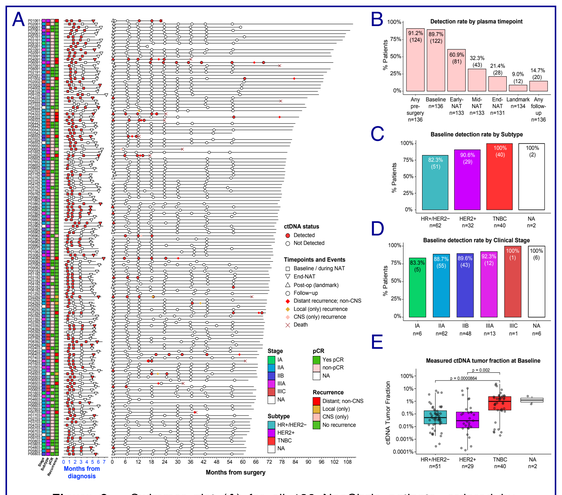

Figure 2 – Swimmer plot (**A**) for all 136 NeoCircle patients, ordered by enrollment date. See key for clinicopathological annotations. ctDNA detection rates by (**B**) plasma timepoint, (**C**) breast cancer subtype, and by (**D**) clinical stage at diagnosis. Measured ctDNA tumor fractions (**E**) at baseline according to breast cancer subtype.

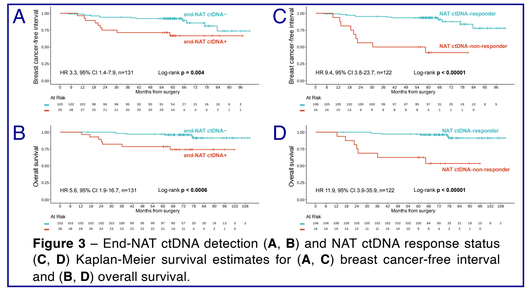

Figure 3 – End-NAT ctDNA detection (**A**, **B**) and NAT ctDNA response status (**C**, **D**) Kaplan-Meier survival estimates for (**A**, **C**) breast cancer-free interval and (**B**, **D**) overall survival.

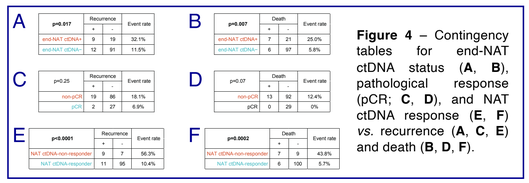

Figure 4 – Contingency tables for end-NAT ctDNA status (**A**, **B**), pathological response (pCR; **C**, **D**), and NAT ctDNA response (**E**, **F**) *vs.* recurrence (**A**, **C**, **E**) and death (**B**, **D**, **F**).

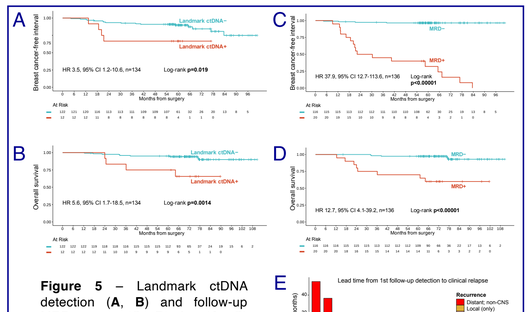

Figure 5 – Landmark ctDNA detection (**A**, **B**) and follow-up MRD status (**C**, **D**) for (**A**, **C**) breast cancer-free interval and (**B**, **D**) overall survival. Lead-time from MRD-positivity to clinical detection of relapse (**E**).

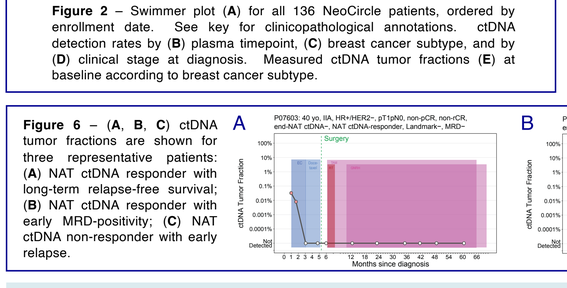

Figure 6 – (**A**, **B**, **C**) ctDNA tumor fractions are shown for three representative patients: (**A**) NAT ctDNA responder with long-term relapse-free survival; (**B**) NAT ctDNA responder with early MRD-positivity; (**C**) NAT ctDNA non-responder with early relapse.

Results:

170 patients with primary stage I-III breast cancer amenable for curative treatment enrolled in the NeoCircle study between December 2014 and March 2019 and plasma samples were collected for ctDNA monitoring.

Results are presented for 1593 plasma samples for 136 patients where minimum QC criteria were met for ctDNA analysis (**Figure 1** and **2**) following a pre-specified analysis plan. The patient tumors comprised 29.4% TNBC, 45.6% HR+/HER2–, and 23.5% HER2+.

- ctDNA was detected in 90% of patients at baseline, at high levels across all breast cancer subtypes and clinical stages. Median variant allele frequency (VAF) at baseline was 0.11% (range 0.000136% - 23.8%) (**Figure 2**).
- ctDNA levels remained detectable at the end of NAT in 21% patients (28/131) and was a significant predictor of poor breast cancer-free interval (BCFi) and overall survival (OS; **Figure 3**).
- Lack of ctDNA response (no decrease or a decrease <50%) during NAT (n=16) significantly predicted very poor BCFi and OS (**Figure 3**).
- End-NAT ctDNA positive (end-NAT+) status and NAT ctDNA non-responder status were associated with significantly higher relapse and death events, whereas non-pathological complete response (pCR) was not (**Figure 4**).
- ctDNA positivity at median day 14 post-operative timepoint (Landmark+) predicted poor BCFi and OS (**Figure 5**). For 8/115 patients without clinical recurrence to date, ctDNA was Landmark+ but subsequently cleared.
- ctDNA positivity at a follow-up timepoint (MRD+) was associated with poor BCFi and OS, and MRD+ provided a median 13.8 months lead-time compared to clinical detection of relapse (**Figure 5**).
- Three representative patient plasma plots are shown (**Figure 6**).

Conclusions:

- In analysis of 136 NeoCircle patients, a study of ctDNA monitoring in early breast cancer patients receiving NAT, we analyzed plasma ctDNA using a novel tumor-informed dPCR assay tracking patient-specific structural variants.
- Ultrasensitive ctDNA detection achieved exceedingly high baseline detection rates across all clinical stages and subtypes.
- ctDNA detection at end of NAT and lack of ctDNA response during NAT associated with high-risk for relapse and death.
- Follow-up MRD detection of ctDNA was associated with poor BCFi and OS and median lead-time of 13.8 months (range 0 to 4 years).
- We validate 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. **4**. Elliott MJ., et al. *Clinical Cancer Research*, 2025. Apr 14;**31**(8):1520-1532.

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