Longitudinal Neoadjuvant and Post-operative Evaluation of Circulating Tumor DNA in Early Breast Cancer Using a Tumor-Informed Assay: Updated Analysis of the TRACER Cohort

**INTRODUCTION**

- Breast cancer is a leading cause of cancer-related death in women worldwide with disease often diagnosed in the early or localized setting.
- Many patients diagnosed with early breast cancer undergo systemic therapy (SCT), usually in the form of multimodality chemotherapy, delivered in a risk-adapted strategy based on routine clinical factors.
- Despite the routine use of radiological and pathologic assessment, full understanding of an individual patient's response to therapy is not fully understood or characterized.
- The assessment of ctDNA and associated therapy-related dynamic changes in breast cancer has been shown to be prognostic. However, the prognostic utility of next-generation highly sensitive assays is currently unknown.
- RaDaR (Neoadjuvant Tumor-informed ctDNA analysis) is a personalized tumor-informed assay capable of detecting ctDNA with high sensitivity and specificity via deep sequencing of up to 10 tumor-specific mutations, identified by tumor- and trait-specific algorithms.
- ctDNA detection with the RaDaR assay after the completion of neoadjuvant therapy is associated with an imminent risk of disease recurrence in high-risk patients (HR+) and triple-negative (TNBC).
- Early data illustrating the performance of the RaDaR assay in the neoadjuvant setting combined with adjuvant surveillance was previously evaluated. Here we present an updated analysis of the TRACER cohort.

**METHODS**

- Participants were enrolled between October 2017 and February 2022 at the University of Toronto (TRACER).
- Plasma samples (1 × 10 ml) were collected at baseline, during treatment, mid-cycle, and at adjuvant follow-up under the IRB-approved study protocol (Figure 1).
- All participants enrolled in TRACER were scheduled to undergo surgery±chemotherapy and were treated with adjuvant systemic therapy.
- Sonar variants were identified through whole-exome sequencing of available sequential formalin-fixed, paraffin-embedded tissue from a diagnostic biopsy or surgical pathology (method described in design personalized familial assay protocol).
- The study cohort was sequenced to identify confirming signals derived from clinical characteristics of the patients and genomic information.
- Personalized ctDNA familial assays were performed on all available plasma samples by Neoradix. ctDNA positivity was defined as presence or absence of a predesigned list of variants.
- Clinical and pathologic characteristics, treatment, and response outcomes were evaluated.
- Recurrence outcomes were not updated on December 1, 2022.

**RESULTS**

- 162 participants were identified from TRACER with 11% enrolled in the primary analysis (Figure 3 and 4).
- ctDNA levels were analyzed from all collected timepoints.
- Median clinical follow-up from diagnosis was 3 years (range: 0.3-6 years).
- A significant drop in ctDNA was observed from diagnosis (median 10% (IQR 50%) to 0% (IQR 0%)), with clear separation of events.
- Median estimated variant allele frequency (vAF) was 0.083% (range: 2.93% to 7.5%).

**CONCLUSIONS**

- RaDaR is a sensitive, tumor-informed assay, which detects ctDNA at baseline in most patients prior to NAT.
- The rate of baseline ctDNA detection varied by receptor subtype and was significantly associated with clinical grade but not other baseline clinical variables.
- ctDNA detection at baseline was prognostic. All participants experiencing disease recurrence had detectable ctDNA at baseline.
- Persistent ctDNA detection measured midway through NAT was prognostic of disease relapse in HR+ and TNBC.
- Clearance of ctDNA was observed with initiation of change in adjuvant systemic therapy but relapse was observed if this was not durable.
- ctDNA can be detected using RaDaR with long lead times prior to clinical recurrence. The clinical utility of monitoring and interventional strategies require prospective evaluation.

**ACKNOWLEDGEMENTS**

- The authors acknowledge the contribution of the TRACER cohort participants, the collaborators, and the team at Neoradix.
- They would like to thank theNeoradix team for their support and contribution to the study.

**REFERENCES**