A personalised sequencing approach for liquid biopsy-based detection of recurrent disease in early-stage breast cancer

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BACKGROUND
- Routine surveillance after primary therapy for early breast cancer (BrCa) is currently limited to imaging.
- Follow-up surveillance using circulating tumour DNA (ctDNA) to detect molecular residual disease (MRD) may be a useful tool for identifying patients who may eventually develop distant metastases and holds promise for earlier intervention and improved overall survival.
- However, such follow-up surveillance requires ultrasensitive ctDNA assays due to the heterogeneous nature of the genomic alterations seen in BrCa.
- Here, we evaluate the clinical utility of RaDaR™ (Figure 1), a personalised sequencing assay for MRD detection and monitoring disease recurrence, in early-stage BrCa patients after standard treatment.

METHODS
- This is a retrospective pilot study on 37 early-stage BrCa patients recruited through the BnRoD Bio registry study (Table 1).
- Somatic variants, identified through whole exome sequencing (WES) of patients’ formalin-fixed, paraffin-embedded (FFPE) tumour tissue obtained from curative-intent surgery, were selected and used in the design of personalised RaDaR assays (38-54 variants/assay; median: 49).
- Plasma samples from 21 patients with confirmed clinical progression (median interval of 18.9 months from primary diagnosis) and 16 case-control patients with no recurrence at the time of 3-years follow-up, were analysed using the corresponding patient-specific RaDaR assay.
- RaDaR data analysis was blinded to disease outcome.

RESULTS
- Of the 16 patients without a documented clinical recurrence, only one patient with a luminal A, stage I tumour was positive for ctDNA.
- ctDNA detection levels in this patient were, however, low (0.0085% VAF), potentially indicating the presence of earlier disease recurrence that precedes clinical progression (Figure 2 and 3C).

CONCLUSIONS
- In this pilot study, the RaDaR assay was able to detect the presence of ctDNA in plasma to levels as low as 0.0029% VAF.
- Results indicate that the sensitive detection of ctDNA is strongly associated with distant recurrence in early-stage BrCa, with 12 of 13 cases being successfully detected (sensitivity of 92%).
- These findings warrant further validation in a larger study population.

ACKNOWLEDGEMENTS
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DISCLOSURES
- The presenting author (Wolfgang Janni; wolfgang.janni@uniklinik- ulm.de) has no conflicts of interest to declare.
- The authors were fully responsible for all content and editorial decisions, were involved in all stages of paper development and have approved the final version.

Table 1. Baseline characteristics of all 37 patients included in the study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients with confirmed clinical recurrence (N = 21)</th>
<th>Patients with no evidence of clinical recurrence (N = 16)</th>
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<tbody>
<tr>
<td>Age at primary diagnosis (years)</td>
<td>Median 60</td>
<td>Range 35–82</td>
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<tr>
<td>Histological grading</td>
<td>G2 14 (66.7%)</td>
<td>G1 0 (0.0%)</td>
</tr>
<tr>
<td>Histological type</td>
<td>Ductal 15 (71.4%)</td>
<td>Lobular 4 (19.0%)</td>
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<tr>
<td>Hormone receptor status</td>
<td>Negative 6 (28.6%)</td>
<td>Positive 15 (71.4%)</td>
</tr>
<tr>
<td>HER2 status</td>
<td>Negative 14 (66.7%)</td>
<td>Positive 0 (0.0%)</td>
</tr>
<tr>
<td>Neoadjuvant chemotherapy</td>
<td>No 14 (66.7%)</td>
<td>Yes 6 (28.6%)</td>
</tr>
<tr>
<td>Median age of primary diagnosis (years)</td>
<td>60</td>
<td>Range 35–82</td>
</tr>
<tr>
<td>Median age of disease recurrence (years)</td>
<td>18.9</td>
<td>Range 11–30</td>
</tr>
</tbody>
</table>

Figure 1. The RaDaR Workflow. Steps involved in the design of personalised RaDaR assays, from WES profiling of a patient’s tumour, to variant identification and selection for panel design and plasma analysis for the detection of molecular residual disease and monitoring for disease recurrence.

Figure 2. Use of personalised RaDaR assays for the detection of recurrent disease in early-stage BrCa patients. cDNA detection in patients with no evidence of disease recurrence (control cases) and in those with clinical confirmation of either local (light red bars) or distant (dark red bars) recurrence. (A) Patient with no documented recurrence and plasma cDNA detected at low levels, (B) Patient with distant recurrence and cDNA not detected, (C) Patient with no documented recurrence and cDNA detected at low levels, (D) Patient with no documented recurrence and ctDNA detected at low levels (estimated VAF: 0.0085%) indicating potential presence of early molecular recurrence (A), Patient with no documented recurrence and cDNA detected, (B) Patient with no documented recurrence and ctDNA detected at low levels (estimated VAF: 0.0085%) indicating potential presence of early molecular recurrence.