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Background and Aim
- Personalized circulating tumor DNA (ctDNA) assay is being evaluated in early breast cancer (BC) setting.
- We investigated ctDNA detection and outcome in early HER2+ breast cancer patients treated with CDK4/6 inhibitors and endocrine therapy.

Methods
- NeoRHEA (NCT03695524) is a single arm phase 2 study in which patients with estrogen receptor (ER)-positive advanced breast cancer were treated with palbociclib plus endocrine therapy (CT) for 4 months.
- Plasma samples were collected at fouroptional timepoints: baseline (BL), after treatment cycle (C1D02), before surgery (Surgery), and one month post-surgery (End of study).
- ctDNA was detected using the personalized RaDaR assay by NanoGenomics Inc.
- Whole-genome sequencing (WES) was performed on BL tumor biopsies followed by a personalized assay development tracking up to 48 patient-specific somatic variants in plasma cfDNA (ctDNA) at ctDNA assessment.
- Associations between ctDNA detection and clinicopathological characteristics of BL were investigated.
- Moreover, associations between ctDNA detection at different time points and clinical outcome measures chosen below were also investigated:
  - Uptake response based on AUC criteria in response with responder defined as patients with complete or partial response while non-responders as patients with stable or progressive disease.
  - Complete clinical response (CCR) defined as M0 at C1D02 and surgery.
  - Residual cancer burden (RCB) (M0, M1, L0, and L1).
- ctDNA detection in circulating tumor DNA (CTD) with (CTD) and without (CTDN) endocrine therapy.

Results - ctDNA detection with RaDaR assay
- Out of the 75 patients, 42 (56%) were found to be CTDI positive at BL. 45 (59%) patients were also CTDI positive at C1D02 with 43 (57%) still CTDI positive at S (4 patients were CTDI positive at S, but not in the earlier C1D02 timing).
- However, none of the patients were CTDI positive at the end of the study.
- 54 patients tested at CTDI positive at all timepoints (always negative, 9 tested positive only at BL (1 patient tested negative at BL and at one or more subsequent timepoints (BL positive at the at least one T1D02 positive).
- RaDaR is calculating the estimated clinical relapse fraction (ECRF) by using a proprietary algorithm the ASSUR BRCM range for the positive CTDI samples was between 0.03% and 0.6% of a median of 0.13%.

Results - BI Clinical pathology characteristics and (CTD) detection at BI
- In our cohort of 18 patients, 57% were postmenopausal (52%). 47% were LumenA (based on IHC and gene expression), 7% were HER2+ (5) and 66% had multifocal/multicentric tumors. 36% had histological grade 3 tumors.
- The BI-CTD detection rates were associated with grade 3 tumors (p < 0.03).
- In contrast, multifocal/multicentric tumors had lower baseline detection rates (p = 0.1).
- No significant associations were found for tumor weight, clinical tumor size, or tumor size.
- We assessed the correlation between BI-CTD percentage and clinicopathological characteristics but did not observe any significant associations.

Results - ctDNA detection and clinical outcome
- Patients grouped by CTDI detection were best fit for association with responses defined as (early) partial or (early) complete responses.
- Grouping patients based on ctDNA monitoring is associated with ultrasound response and CDRB, marked with M0 at S.

Table 1. RaDaR assay metrics

<table>
<thead>
<tr>
<th>Metric</th>
<th>Median</th>
<th>Interquartile range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum extracted ctDNA volume</td>
<td>0.266</td>
<td>0.08-0.52</td>
</tr>
<tr>
<td>Minimum total copies</td>
<td>780,000</td>
<td>277,000-2,694,000</td>
</tr>
<tr>
<td>Minimum RaDaR input DNA</td>
<td>0.266</td>
<td>0.08-0.52</td>
</tr>
<tr>
<td>Total variants selected</td>
<td>48 (18)</td>
<td>29 (17-47)</td>
</tr>
<tr>
<td>Total variants that passed QC</td>
<td>10 (7)</td>
<td>10 (7)</td>
</tr>
</tbody>
</table>

Table 2. The table contains the first column of the patients group definition and all the other columns contain the respective measurements. On the row, the number of patients in each group and each response category is shown. The last row is presenting the patients' exact test p-values for all groups per clinical outcome.

<table>
<thead>
<tr>
<th>Patients group</th>
<th>BL-RS Low</th>
<th>BL-RS High</th>
<th>Ultrasound Response</th>
<th>Ultrasound Non-response</th>
<th>M0 at S</th>
<th>M0 at D02</th>
<th>M0 at D02</th>
<th>M0 at D02</th>
<th>M0 at D12</th>
<th>M0 at D12</th>
<th>M0 at D12</th>
</tr>
</thead>
<tbody>
<tr>
<td>AlwayNegative (n = 34)</td>
<td>26 6 17</td>
<td>17 17 17</td>
<td>10 3 0</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>BlusonicThermPositive (n = 36)</td>
<td>20 15 22</td>
<td>13 21 23</td>
<td>3 0 0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>BlusonicThermPositive (n = 5)</td>
<td>2 3 0</td>
<td>2 2 2</td>
<td>0 0 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

Conclusions
- Our data suggests association of ctDNA with pathological and clinicopathological parameters.
- CTDI detection after one month of treatment with Palbociclib and ET was associated with worse outcome.
- Independent validation is needed.

Acknowledgements
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References