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Circulating tumor DNA monitoring in patients with breast cancer receiving neoadjuvant palbociclib and endocrine therapy: A secondary analysis of the NeoRHEA phase 2 study.

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Background and Aim

- **Personalized circulating tumor DNA (ctDNA)** assays are being evaluated in **early breast cancer** (BC) [1].
- We investigated **ctDNA detection** and **outcome** in early HR+/HER- breast cancer patients treated with **CDK4/6 inhibitors and endocrine therapy**.

Methods

- **NeoRHEA (NCT03065621)** is a single arm phase 2 study in which patients with estrogen receptor (ER)+/HER2- early breast cancer were treated with neoadjuvant palbociclib plus endocrine therapy (ET) for 4 months.
- Plasma samples were collected at four timepoints: baseline (BL), after treatment cycle (C1D28), before surgery (Surgery) and one month post Surgery (End of Study).
- ctDNA detection was evaluated using the personalized RaDaR® assay by NeoGenomics Inc. Whole-exome sequencing (WES) was performed on BL tumor biopsies followed by a personalized assay development tracking up to 48-patient specific somatic variants in plasma cell-free DNA (cfDNA) using next generation sequencing.
- Associations between ctDNA detection and clinicicopathological characteristics at BL were investigated.
- Moreover, associations between ctDNA detection at different time points and clinical outcome measures shown below were also investigated:
  1. Ultrasound response based on WHO criteria evaluation with responders defined as patients with complete or partial response while non-responders as patients with stable or progressive disease,
  2. Complete cell cycle arrest (CCCA) defined as Ki67 ≤2.7% at surgery (Ki67 at SG),
  3. Residual cancer burden (RCB) 0/I/II (Low) vs III (High) and
  4. Breast cancer free survival (BCFS) with events being locoregional or distant relapses

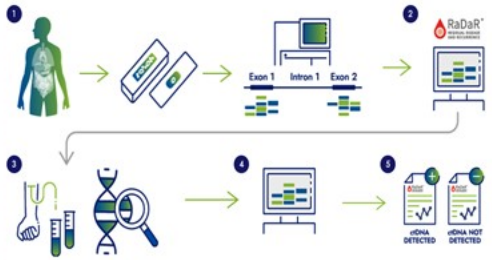

Figure 1. RaDaR workflow: WES is performed on tumor biopsy and a list of mutations are selected. Plasma cell free DNA and whole blood cells(WBC) are collected and targeted sequencing is performed on plasma cell free DNA using the detected tumor mutations. [2]

Results - RaDaR metrics

- The NeoRHEA study enrolled 100 patients, of which 80 patients and 313 plasma samples were selected for RaDaR testing. Among these 80 patients, **78 patients and 302 plasma samples** met the QC thresholds and were successfully profiled using the RaDaR assay.

Assay Metrics	Median (Min. - Max.)
Median Extracted plasma volume	3.36uL (0.55 - 5.23)
Median Total Extracted Copies	6760 (884 - 276,640)
Median RaDaR Input DNA	5,200 (680 - 20,000)
Total variants selected	48 (18 - 52)
Total variants selected that passed QC	25 (7 - 48)

Table 1. RaDaR assay metrics

Results – ctDNA detection with RaDaR assay

- Out of the 78 patients, 42 (53%) **were found to be ctDNA positive at BL**, 4 (5%) patients were also ctDNA positive at C1D28 with 3 of them still ctDNA positive at SG. A fourth patient was ctDNA positive at SG, but not in the earlier C1D28 timepoint. However, none of the patients were ctDNA positive at the end of the study.
- 34 patients tested ctDNA negative at all timepoints (AlwaysNegative), 35 tested positive only at BL (BLpositiveThenNegative) and 5 tested positive both at BL and at one or more subsequent timepoints (BLpositiveThenPositive).
- RaDaR is calculating the estimated variant allele fraction (eVAF) using a proprietary algorithm; the **%eVAF range** for the positive ctDNA samples was between **9.93E-04 and 9.12E-01** with a median of 2.63E-02.

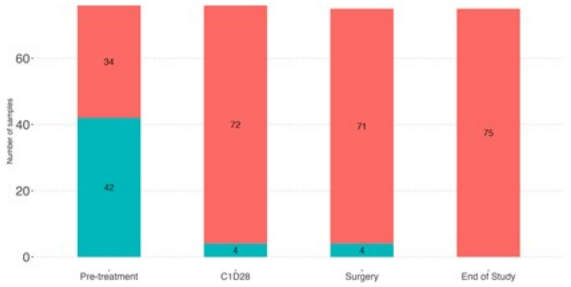

Figure 2. ctDNA detection by timepoints. Y-axis represents the number of patients analysed and X-axis the four timepoints tested. Each number on the bars represents the absolute number of patients in the specific category (TRUE - ctDNA detection / ctDNA FALSE - no ctDNA detection)

Results – BL Clinicopathological characteristics and ctDNA detection at BL

- In our cohort of 78 patients, 67% were postmenopausal (52), 47% were Luminal A (based on PAM50 gene expression), 75 % had cT2 (59) and 69% cN0 tumors(54), 20% had multifocal/multicentric tumors, 16% had histological grade 3 tumors .
- BL ctDNA detection rates were associated **with grade 3 tumors** (p=0.03). In contract, multifocal/multicentric tumors had lower baseline detection rates (p=0.01)
- No significant associations were found for menopausal status, clinical nodal status nor clinical tumor size
- We assessed the correlation between BL %eVAF percentage and clinical/pathological characteristics but did not observe any significant associations.

Results – ctDNA detection and clinical outcome (I)

- Patients grouped by ctDNA detection were tested for association with response(as defined previously) using Fisher's exact test
- Grouping patients based on ctDNA monitoring is associated with Ultrasound response and RCB class, but not with Ki67 at SG

Patients Group	RCB Low	RCB high	Ultrasound Responder	Ultrasound Non-responder	Ki67 at SG <=2.7%	Ki67 at SG >=7.4%	Ki67 at SG Indecisive
AlwaysNegative (n = 34)	28	6	17	17	20	3	5
BLpositiveThenNegative (n = 35)	20	15	22	13	21	3	0
BLpositiveThenPositive (n = 5)	2	3	0	5	2	2	0
Fisher's exact test p-value	0.0241		0.024		0.0508		

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

Results – ctDNA detection and clinical outcome (II)

- With a median follow-up of **3.8 years** (range 1-5 years), 4 patients developed distant and one patient locoregional recurrences.
- ctDNA detection after one month of treatment (logrank p=0.02) was **associated with worse BCFS**, but not at baseline nor at surgery (p=0.59, p=0.67)

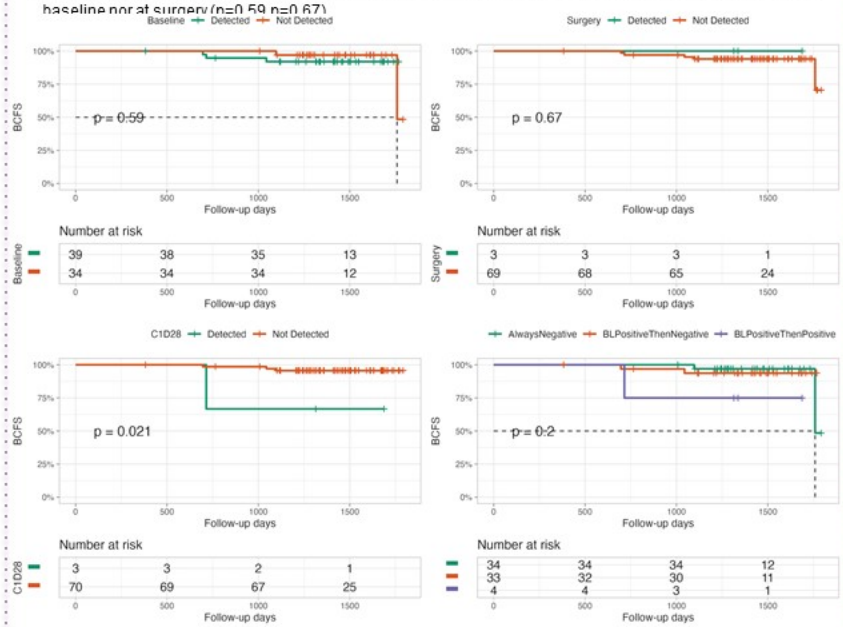

Figure 3 - Kepler Meier curves with BCFS events for the three timepoints (BL, C1D28 and Surgery) and for the groups defined in Table 2

Conclusions

- Our data **suggests association of cDNA detection** with pathological and clinical variables.
- CtDNA detection after one month of treatment with Palbociclib and ET was associated with worse outcome
- Independent validation is needed.

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