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 BRandO 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.

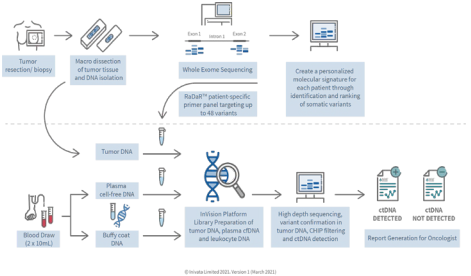

Figure 1. The RaDaR Workflow. Steps involved in the design of personalised RaDaR assays, from WES profiling of a patient's tumour, to variant identification and selection for panel design and plasma analysis for the detection of molecular residual disease and monitoring for disease recurrence.

RESULTS

Study Cohort

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

Variable		Patients with confirmed clinical recurrence (N = 21)	Patients with no evidence of clinical recurrence (N = 16)
Age at primary diagnosis (years)	*Median*	62	60.5
	Range	35 - 82	31 - 83
Histological grading	*G1*	0 (0.0%)	0 (0.0%)
	G2	14 (66.7%)	14 (87.5%)
	G3	7 (33.3%)	2 (12.5%)
Histological type	*Ductal*	15 (71.4%)	12 (75.0%)
	Lobular	4 (19.0%)	4 (25.0%)
	Other	2 (9.5%)	0 (0.0%)
Hormone receptor status	*Negative*	6 (28.6%)	1 (6.3%)
	Positive	15 (71.4%)	15 (93.8%)
HER2 status	*Negative*	20 (95.2%)	14 (87.5%)
	Positive	1 (4.8%)	2 (12.5%)
Neoadjuvant chemotherapy	*No*	14 (66.7%)	11 (68.8%)
	Yes	6 (28.6%)	4 (25.0%)
	Unknown	1 (4.8%)	1 (6.3%)

ctDNA detection is strongly associated with distant recurrence in early-stage BrCa patients

- ctDNA was detected in 15 of 21 patients with confirmed clinical recurrence (71%) at an estimated median variant allele frequency (VAF) of 0.827% (range: 0.0029% to 37.8%).
- When ctDNA detection was evaluated by type of recurrence, 12/13 patients with distant disease were ctDNA positive (92%) compared to 3/8 patients with local recurrence (38%) (**Figure 2**).
- Patients with distant recurrence had the highest plasma ctDNA levels (estimated median VAF: 6.947%, Range: 0.0276% to 37.8%) (**Figure 3A**)
- The lowest ctDNA levels were seen in the 3 patients with local recurrence (0.0029%, 0.0146% and 0.0248%; **Figure 2 and 3B**).
- Of the remaining 6 patients with clinically confirmed recurrence but no detectable ctDNA levels, 5 had local and one distant recurrence.
- Pathological review of the ctDNA negative distant recurrence specimen (ovary) revealed an unusual histology, when compared to the primary breast tumour, indicative of either an alternative origin or a second primary tumour.

- 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 early molecular recurrence that precedes clinical progression (**Figure 2 and 3C**).

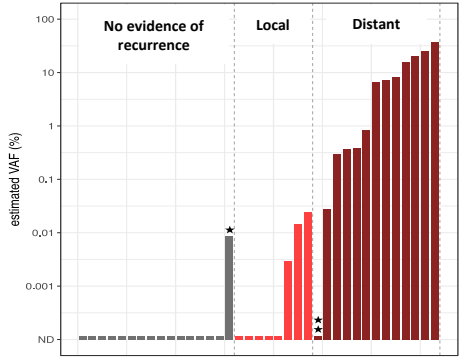

Figure 2. Use of personalised RaDaR assays for the detection of recurrent disease in early-stage BrCa patients. ctDNA detection in patients with no evidence of disease recurrence (control cases) and in those with clinical confirmation of either local (light red bars) or distant (dark red bars) recurrence. (★) Patient with no documented recurrence and plasma ctDNA detected at low levels, (★★) Patient with distant recurrence and ctDNA not detected. **ND**, No detection.

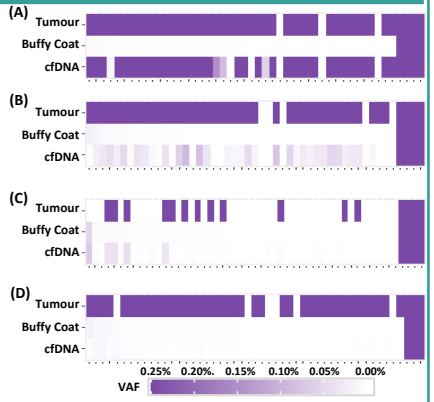

Figure 3. ctDNA detection heatmaps. Each column represents a different WES-derived variant, each row a different sample type (tumour DNA, buffy coat and plasma) analysed by RaDaR. Variants present in the buffy coat are identified as germline or CHIP variants and are excluded from the analysis, as well as variants that are not confirmed in the tumour specimens. (**A**) Patient with distant recurrence showing high ctDNA plasma levels (estimated VAF: 25.4%) (**B**) Detection of ctDNA at an estimated VAF of 0.0248% in a patient with local recurrence (**C**) Patient with no documented recurrence and ctDNA detected at low levels (estimated VAF: 0.0085%) indicating potential presence of early molecular recurrence (**D**) A patient with no documented recurrence and ctDNA not detected.

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.

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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 poster development and have approved the final version.