Detection of early-stage breast cancer recurrence using a personalized liquid biopsy-based sequencing approach

Wolfgang Janni[1], Jens Huober[1], Sophia Huesmann[1], Christodoulos Pipinikas[2], Tatjana Braun[1], Volkmar Müller[3], Giovanni Marsico[2], Angelina Fink[1], Paula Freire-Pritchett[2], Karin Koretz[4], Charlene Knape[2], Amelie de Gregorio[1], Brigitte Rack[1], Thomas WP Friedl[1], Lisa Wiesmüller[1], Peter Möller[4], Karen Howarth[2], Klaus Pantel[5], Nitzan Rosenfeld[2]

[1] Department of Obstetrics and Gynecology, University Hospital Ulm, Ulm, Germany; [2] Inivata Ltd, Babraham Research Park, Cambridge, United Kingdom; [3] Department of Obstetrics and Gynecology, University Hospital Hamburg, Hamburg, Germany; [4] Department of Pathology, University Hospital Ulm, Ulm, Germany; [5] Department of Tumour Biology, University Hospital Hamburg, Hamburg, Germany

December 7-10, 2021

#768

Background

- Detection of minimal residual disease (MRD) using circulating tumour DNA (ctDNA) represents an attractive alternative to imaging, currently considered the gold standard in routine surveillance for early breast cancer (BrCa) following primary therapy .
- ctDNA has the potential to identify patients who may eventually develop distant metastatic disease and as such, its implementation in the routine clinical follow-up setting may offer the means for earlier intervention for patients with oligometastatic disease and improved overall survival.
- Due to the highly heterogeneous nature of genomic alterations seen in BrCa, ultra-sensitive ctDNA detection assays are required for follow-up surveillance.
- Here we evaluate RaDaR™ (**Figure 1**), a personalized multiplex PCR-based NGS assay for MRD detection and monitoring disease recurrence in early-stage BrCa patients after standard treatment.

Methods

- A total of 39 early-stage BrCa patients (18% TNBC, 74% HR+/HER2-, 8% HER2+) recruited through the BRandO BiO registry were included in this retrospective pilot study (**Table 1**).
- 21 patients (54%) experienced clinical recurrence (13 distant and 8 local) with a median time to progression of 18.9 months.
- 18 (46%) case-control patients remained recurrence-free at the time of 3-year follow up.
- Personalized RaDaR™ panels designed to target selected somatic variants identified through WES of patients' FFPE tumor tissue from curative-intent surgery (38-54 variants/panel; median: 49) were used to analyze a total of 53 samples taken at:
  - Time of recurrence and, where available, at 12-months post-diagnosis (33 samples from 21 patients).
  - 3-years follow-up in cases with no confirmed clinical recurrence, including one control case with additional samples analyzed at 12-months and 4-years of follow up (20 samples from 18 patients).

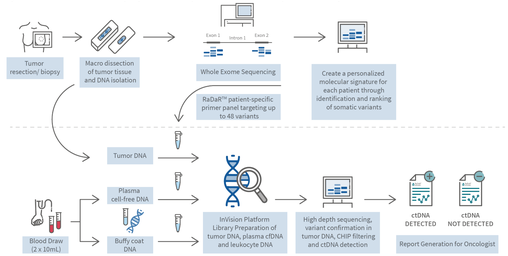

Figure 1. The RaDaR Workflow. Steps involved in the design of personalised RaDaR assays, from WES profiling of a patient's tumour, to variant identification and selection for panel design and plasma analysis for the detection of molecular residual disease and monitoring for disease recurrence.

Study Cohort

Table 1. Baseline characteristics for the enitre study cohort

		Patients with confirmed clinical recurrence (*N*=21)	Patients with no evidence of clinical recurrence (*N*=18)
Age at primary diagnosis (years)	Median	62	59
	Range	35 - 82	31 - 83
Histological grading	G1	0 (0.0%)	0 (0.0%)
	G2	14 (66.7%)	16 (88.9%)
	G3	7 (33.3%)	2 (11.1%)
Tumor stage	*pT1*	4 (19.0%)	4 (22.2%)
	pT2	8 (38.1%)	7 (38.9%)
	pT3	3 (14.3%)	1 (5.6%)
	pT4	0 (0.0%)	1 (5.6%)
	*Unknown**	6 (28.6%)	5 (27.8%)
Histological type	*Ductal*	15 (71.4%)	14 (77.8%)
	Lobular	4 (19.0%)	4 (22.2%)
	other	2 (9.5%)	0 (0.0%)
Hormone receptor status	*Negative*	6 (28.6%)	1 (5.6%)
	Positive	15 (71.4%)	17 (94.4%)
HER2 status	*Negative*	20 (95.2%)	16 (88.9%)
	Positive	1 (4.8%)	2 (11.1%)
Neoadjuvant chemotherapy	*No*	14 (66.7%)	13 (72.2%)
	Yes	6 (28.6%)	4 (22.2%)
	Unknown	1 (4.8%)	1 (5.6%)

* Neoadjuvant therapy/no surgery

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

- ctDNA was detected in 15/21 (71%) patients with confirmed clinical recurrence at an estimated median variant allele frequency (eVAF) of 0.827% (range: 0.0029% to 38%).
- 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 (median eVAF: 6.56%, 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% eVAF; **Figure 2 and 3B**).
- Of the 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, indicating a possible alternative origin or second primary tumor.
- Of the 18 case-control patients, only one with a Luminal A, stage I tumor was positive for ctDNA potentially indicating the presence of early molecular recurrence that precedes clinical progression (**Figure 3C and 3D**). Two additional timepoints from this patient were also positive for ctDNA, which could be indicative of residual disease remaining dormant (**Figure 4A**).

Results

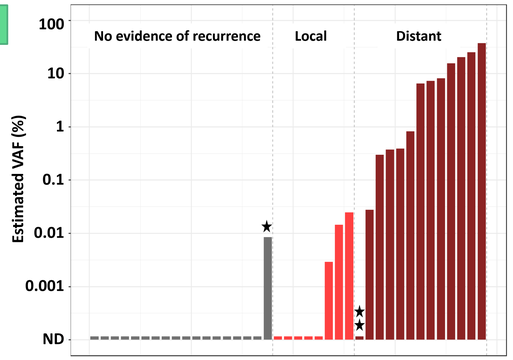

Figure 2. Use of personalized RaDaR assays for the detection of recurrent disease in early-stage BrCa patients. ctDNA detection in patients with no evidence of disease recurrence (control cases) and in those with clinical confirmation of either local (light red bars) or distant (dark red bars) recurrence.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

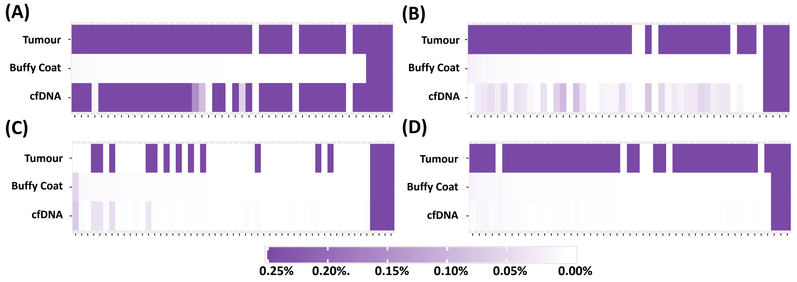

Figure 3. ctDNA detection heatmaps. Each column represents a WES-derived variant, each row a different sample type (tumour DNA, buffy coat and plasma) analyzed by RaDaR. Variants present in buffy coat are identified as germline or CHIP variants and are excluded from the analysis, as well as variants that are not confirmed in the tumour specimen. (**A**) Patient with distant recurrence showing high ctDNA plasma levels (eVAF: 25.4%) (**B**) Detection of ctDNA in a patient with local recurrence at an estimated VAF of 0.0248% (**C**) Control patient with low plasma ctDNA levels (eVAF: 0.0085%) indicating potential presence of early molecular recurrence (**D**) A control patient negative for ctDNA.

- An earlier plasma sample was available for 12 patients with disease recurrence (5 distant & 7 local recurrence)
- 4 patients (3 with distant and one with local recurrence) had ctDNA detected at this earlier timepoint with a median lead time of 92 days (range: 42-308 days) from ctDNA detection to confirmed clinical recurrence.
- Three patients had ctDNA detected only at the time of recurrence (one distant and 2 local recurrence).
- None of the 5 patients with negative ctDNA results at the time of recurrence had detectable ctDNA levels at this earlier timepoint (1 distant and 4 local recurrence)
- **Figure 4B** shows an example of ctDNA detection 308 days ahead of clinical recurrence.

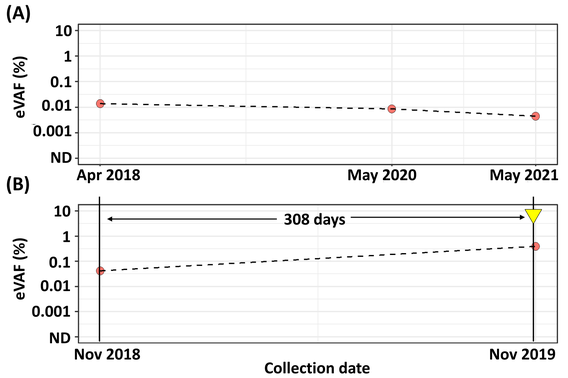

Figure 4. Longitudinal ctDNA detection using personalized RaDaR assays. (A) Persistent ctDNA detection at three timepoints (12-months, 3-years and 4-years post-diagnosis) in a patient with no confirmed clinical recurrence (indicative of residual disease remaining dormant). **(B)** Detection of ctDNA 12-months post-diagnosis, 308 days ahead of clinical confirmation of disease relapse (Nov 2019) (marked with an inverted yellow triangle).

Conclusions

- In this real-world pilot study, the RaDaR™ assay detected the presence of ctDNA in plasma to levels as low as 0.0029% eVAF.
- We have shown that presence of ctDNA is strongly associated with distant recurrence in early-stage BrCa with a detection sensitivity of 92% (12 of 13 cases detected).
- In a limited number of cases where samples were available prior to recurrence, ctDNA could be detected ahead a clinical progression.
- This potentially offers the opportunity for earlier intervention aiming at improving overall survival rates and will be tested prospectively through the German SURVIVE study.

Acknowledgements

The authors would like to acknowldedge the following:
- All patients and their families for agreeing to participate in the study.
- Inivata's Product Development, Computational Biology and Clinical Operations teams.

Disclosures

- The presenting author 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.