Molecular Residual Disease detection in early stage breast cancer with a personalized sequencing approach

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Background

Detection of circulating tumor DNA (ctDNA) presents a strategy to identify Molecular Residual Disease (MRD) in patients with breast cancer. Tools capable of detecting ctDNA at lower concentrations are needed to increase sensitivity and lengthen lead time between ctDNA detection and relapse. We present results from a highly sensitive personalized sequencing approach for ctDNA detection of MRD based on multiple patient specific mutations.

Methods

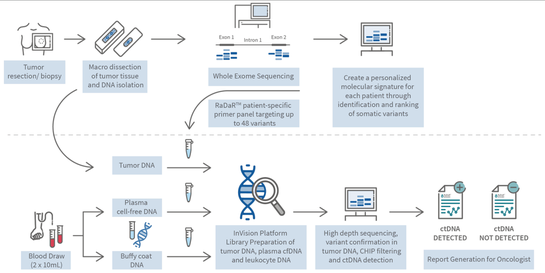
- 22 early breast cancer patients (12 HR+HER2-, 7 HER2+ and 3 TNBC) enrolled in the ChemoNEAR sample collection study were included.

- Tumor DNA from FFPE samples was Whole Exome Sequenced to identify patient specific mutations and design personalized Residual Disease and Recurrence (RaDaR^{TM}) multiplex PCR NGS assays.

- Cell free DNA was extracted from 147 plasma samples (median volume 4ml, range 0.5-5ml) and sequenced with RaDaR^{TM} assays, with 10-61 variants (median 41) per panel, to 100,000x per locus. A matched single timepoint buffy coat was sequenced to verify removal of germline SNPs and any potential clonal hematopoiesis (CHIP). A proprietary algorithm was used to identify ctDNA. Droplet digital PCR (ddPCR) was performed as previously described[1,2].

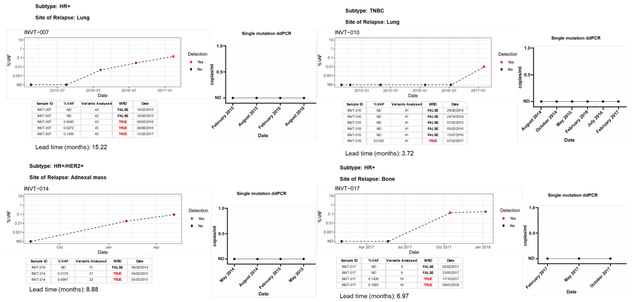
- Tumor Sequencing of multiple biopsy timepoints was carried out for 14 patients (mean 2.8 samples per patient) and clonal populations estimated with Pyclone. For clusters of greater than 10 mutations, RaDaR^{TM} panels were supplemented with additional variants for clonal tracking.

RaDaR^{TM} assay workflow

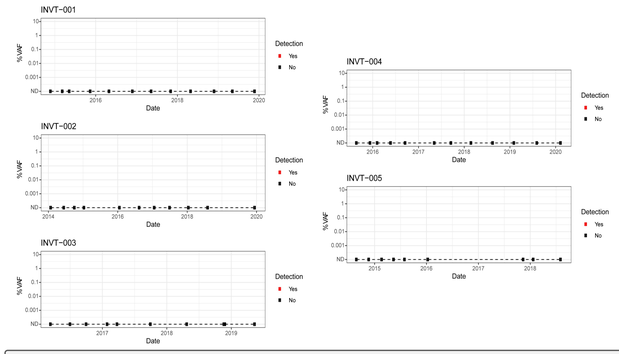


MRD detection with personalized sequencing panels

MRD was identified in 100% (17/17) of relapsed patients (p=0.0002, Log rank test). Detection of ctDNA levels ranged from 0.0007% estimated Variant Allele Frequency (VAF) to 1.3% VAF (median 0.06% VAF). Five relapsed patients (4 extracranial and 1 brain only) had MRD detected with their corresponding RaDaR^{TM} assays but not by single gene ddPCR.

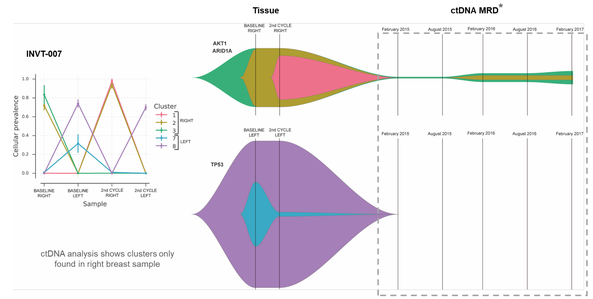


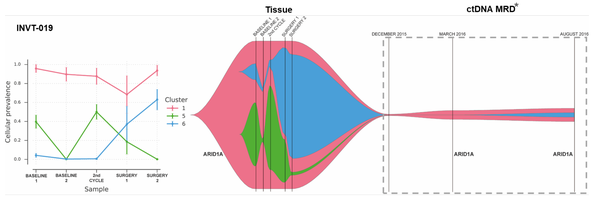
MRD was not identified in 54 time points (range 37.29 months to 69.22 months) in the 5 patients that did not relapse (p=0.0002, Log rank test).

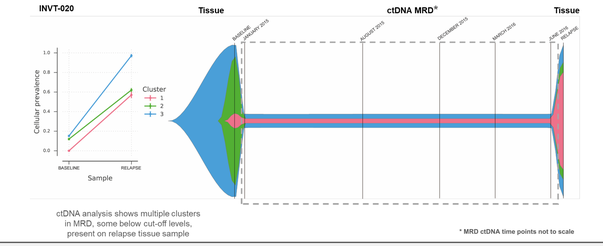


Tracking clonal evolution in the MRD Setting with ctDNA

In 8/14 patients with multiple tumor samples sequenced, multiple clones (mean 3.4 clones/patient) were identified, with heterogeneous polyclonal relapse in 4/8 patients, and a single clone detectable in 4/8 patients.

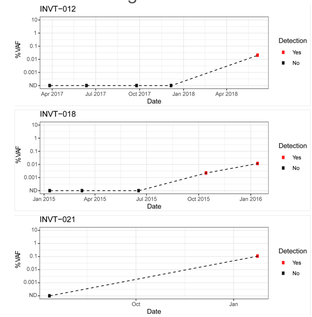






Brain only relapses detected by RaDaR^{TM} assay

In three patients with brain only relapse, ctDNA was detected prior to relapse in all patients (3/3, 100%) albeit with a reduced lead time over clinical relapse (3.85, 4.21 and 5.65 months), which was not previously achievable with single mutation ddPCR MRD-detection assays.

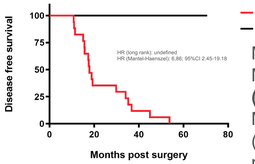


Sample ID	%VAF	Variants Analysed	MRD	Date
INVT-012	ND	21	FALSE	23/03/2017
INVT-012	ND	21	FALSE	13/06/2017
INVT-012	ND	21	FALSE	26/09/2017
INVT-012	ND	21	FALSE	07/12/2017
INVT-012	0.0206	21	TRUE	06/06/2018

Sample ID	%VAF	Variants Analysed	MRD	Date
INVT-018	ND	48	FALSE	12/01/2015
INVT-018	ND	48	FALSE	10/03/2015
INVT-018	ND	48	FALSE	18/06/2015
INVT-018	0.0022	48	TRUE	15/10/2015
INVT-018	0.0118	47	TRUE	14/01/2016
INVT-018	0.0034	48	TRUE	20/11/2017
INVT-018	0.0007	48	TRUE	30/07/2018
INVT-018	0.0078	48	TRUE	28/12/2018

Sample ID	%VAF	Variants Analysed	MRD	Date
INVT-021	ND	49	FALSE	12/07/2013
INVT-021	0.1088	49	TRUE	24/01/2014

Detection of ctDNA Post Surgery is Associated with Higher Risk of Relapse



Median Follow-up: **24.6** months.
Median Lead Time to clinical Relapse: **8.88 (7.08-9.96)** months.
Median Lead Time to clinical relapse (extracranial only): **12.89 (11.8-14.02)** months.

Conclusions

In a proof-of-principle study, ctDNA-detected MRD with personalized sequencing assays associates with relapse free survival and long lead time over clinical relapse in early stage breast cancer. Sequencing based ctDNA testing can detect patients with brain-only relapses and increases sensitivity over first generation ddPCR-based ctDNA assays. Clonal shifts between primary and recurrence can be anticipated at molecular relapse.

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References

1. Garcia-Murillas *et al.*, STM (2015)
2. Garcia-Murillas *et al.*, JAMA Onc (2019)