Ultra-sensitive ctDNA mutation tracking to identify molecular residual disease and predict relapse in early breast cancer patients

Isaac Garcia-Murillas, Rosalind J Cutts, Charles W Abbott, Sean M Boyle, Jason Pugh, Richard O Chen, Kathryn Dunne, Catey Bunce, Stephen RD Johnston, Alistair Ring, Simon Russell, Abigail Evans, Anthony Skene, Duncan Wheatley, Ian E Smith, Nicholas C Turner

PRESENTED BY: Isaac Garcia-Murillas, PhD

Learning outcomes

- Ultrasensitive detection with a bespoke whole genome sequencing based tracking assay improves detection of ctDNA at baseline and during follow-up and increases lead time over clinical relapse

- ctDNA detection during follow-up strongly associates with worse relapse free and overall survival

- An ultrasensitive detection assay identifies early MRD+ patients that clear ctDNA and do not relapse during long follow-up, with the biology of this currently not understood

Background: ctDNA detection in early breast cancer

- Detection of circulating tumor DNA (ctDNA) in patients with early-stage breast cancer, after completion of curative intent therapy, associates strongly with relapse

- To enable the detection of clinically occult molecular residual disease (MRD), assays with very high sensitivity to detect very low levels of ctDNA, are required

- Genotyping assays currently used in the advanced-stage setting lack the required sensitivity and specificity to detect ctDNA in early-stage settings

- Current MRD tumor informed assays use exome sequencing to identify mutations to track in plasma DNA

Background: ctDNA detection in early breast cancer

With current generation of exome powered MRD assays:

- ctDNA detection rates at diagnosis prior to treatment: **51%-84%**
- Lead time from molecular relapse to clinical relapse: **8.9-11.7 months**

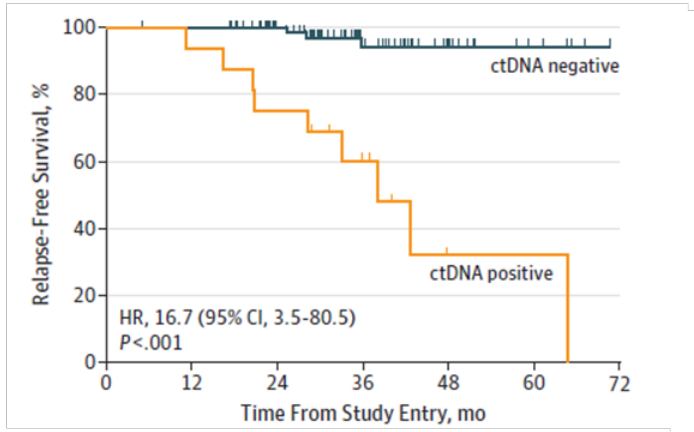
Digital PCR

Multi-mutation sequencing

Molecular Relapse

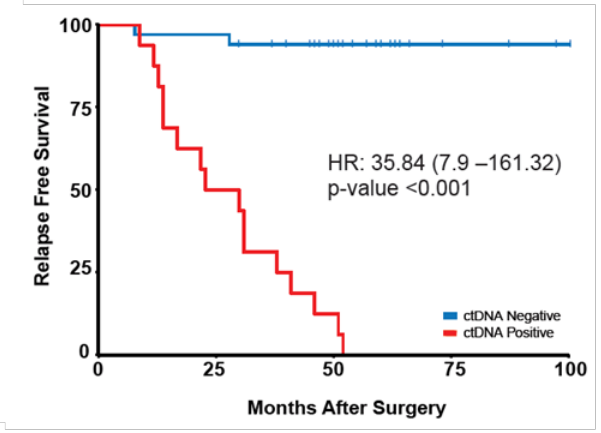
Up to 1-2 mutations

n=101; All Subtypes



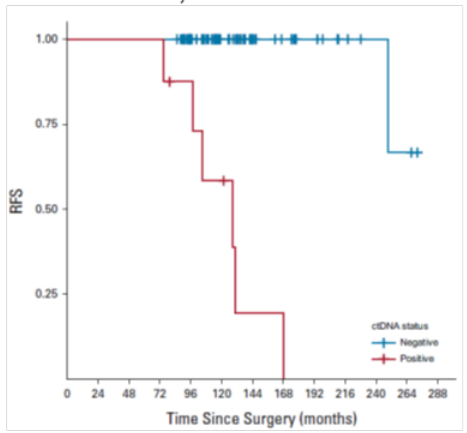
Up to 16 mutations

n=49; All Subtypes



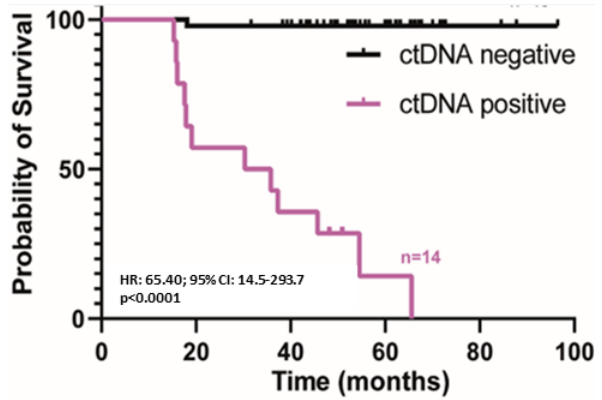
Up to 48 mutations

n=83; HR+/HER2-



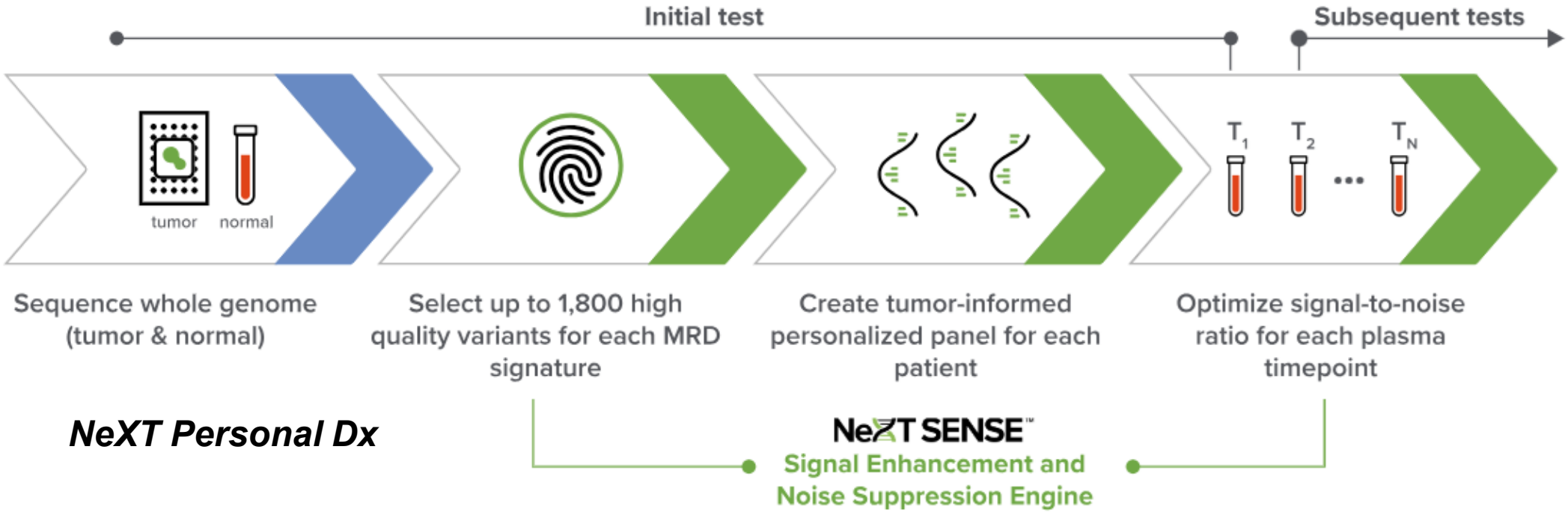
Up to 50 mutations

n=62; All subtypes



Garcia-Murillas *et al.*, JAMA Oncol 2019; Coombes *et al.*, CCR 2019; Cailleux *et al.*, JCO Pres Onc 2022; Garcia-Murillas *et al.*, SABCS 2022 Meeting; Lipsyc-Sharf *et al.*, JCO 2022; Magbanua *et al.*,2023 Cancer Cell

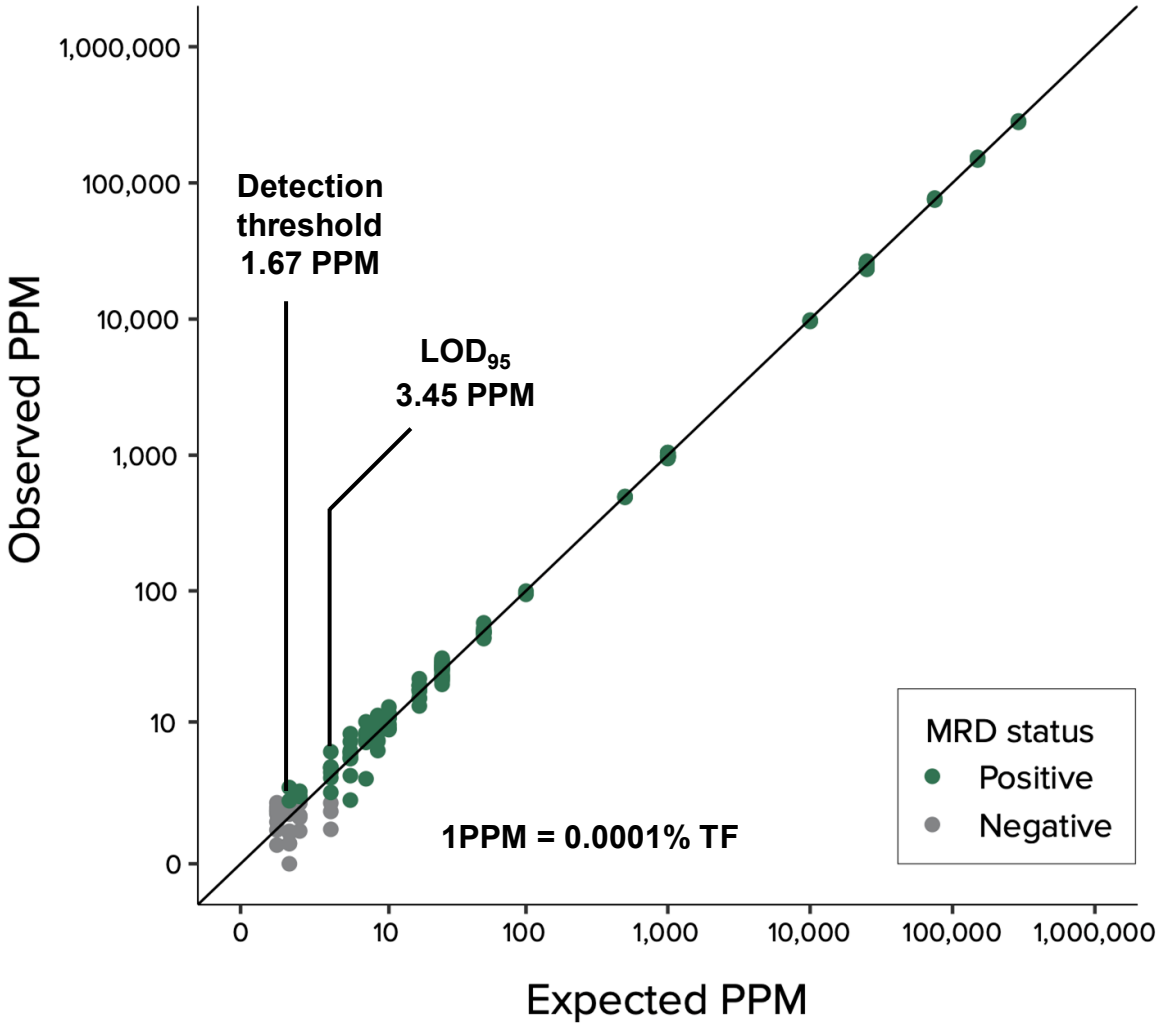
Tumor informed Whole Genome powered MRD detection



Matched tumor FFPE DNA/germline samples were WGS sequenced to a median depth of **38x** (tumor range 25.3 - 37.5; normal range 38.1- 40.7)

cfDNA was extracted from a median volume of **3.3 ml** of plasma (range 1-4.8 ml) and panels designed contained a median of **1,421 variants/panel** (range 706-1,934/panel)

Ultrasensitive MRD detection down to 1 Part Per Million (PPM)



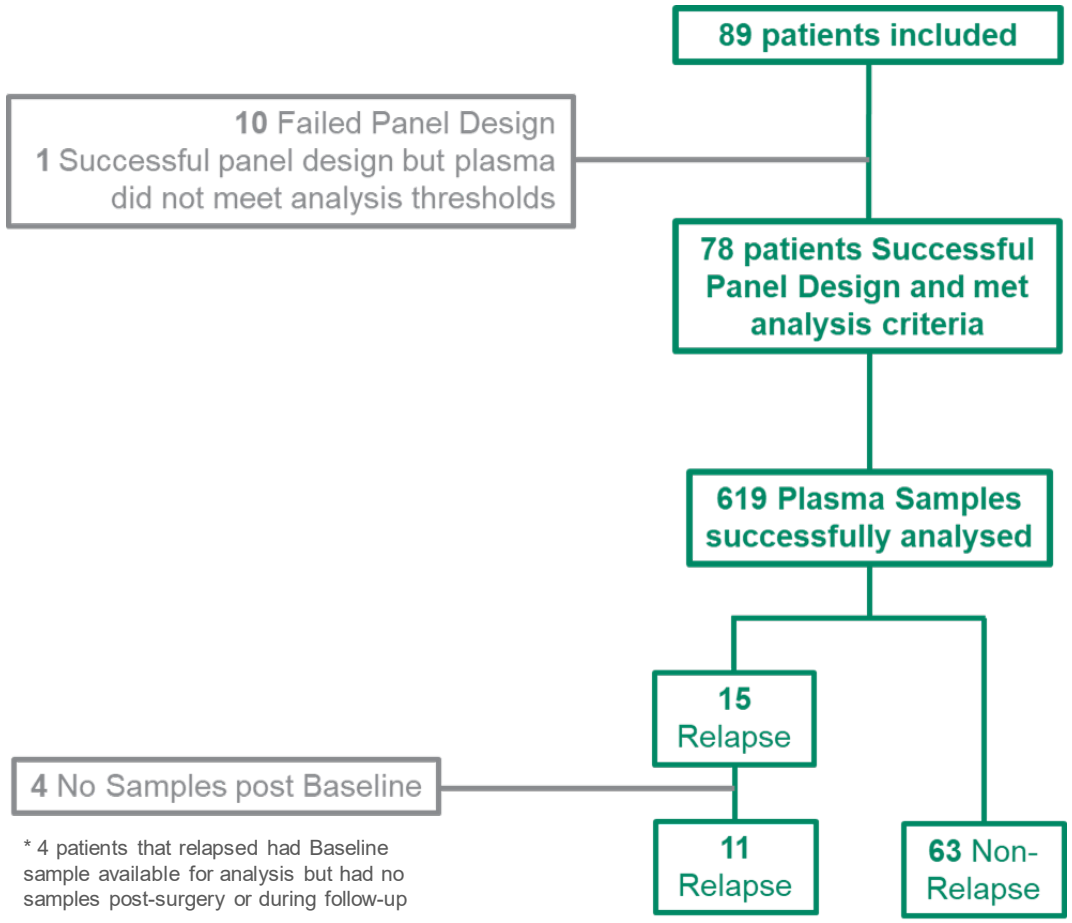
95% Limit of detection (LOD_{95}): **3.45 PPM**

The ctDNA level which can be detected in 95% of assay repeats

Specificity **>99.9%**

Northcott *et al., Oncotarget 2024*

Methods: Patient Cohort and Characteristics

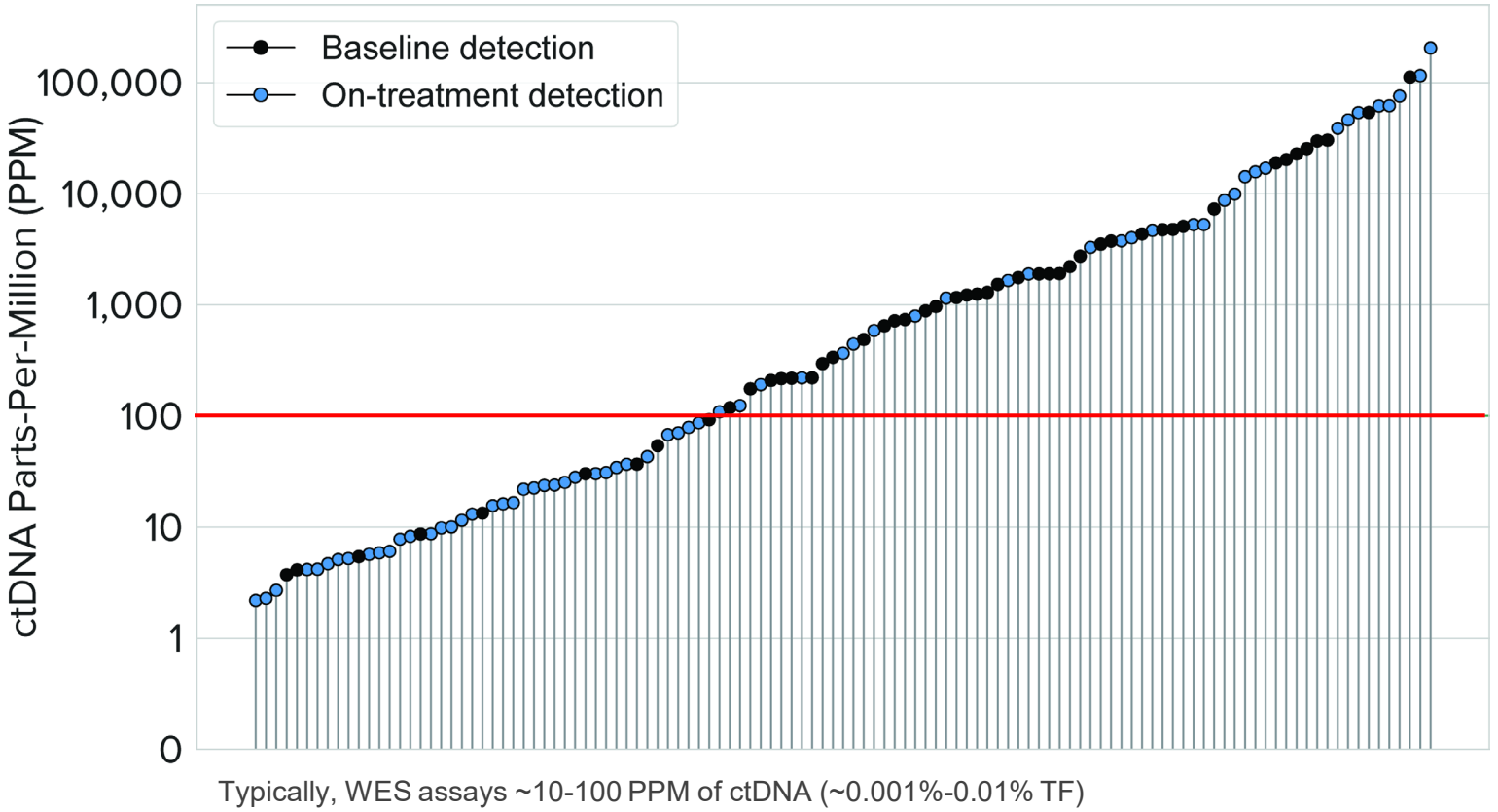


Median:
8 samples/patient
(range 2-14)

Median Follow-up from study entry was **76** months (5-113 months)

N		78 (100%)
Median Age at Diagnosis		50 (25-77)
Menopausal status	*Pre*	44 (56.4%)
	Post	29 (37.2%)
	Not Know	5 (6.4%)
Nodal Status	*Y*	41 (52.6%)
	N	34 (43.5%)
	Not Know	3 (3.9%)
Histological Subtype	*IDC*	65 (83.3%)
	ILC	4 (5.1%)
	Invasive	7 (9%)
	Not known	2 (2.6%)
Tumor Grade	*2*	13 (16.7%)
	3	61 (78.2%)
	Not Know	4 (5.1%)
Receptor Subtype	*HR+ HER2-*	18 (23.1%)
	HER2+	35 (44.8%)
	TNBC	23 (29.5%)
	Not Know	2 (2.6%)
pCR	*Y*	30 (38.4%)
	N	46 (59%)
	Biopsy NA	2 (2.6%)
Neoadjuvant	*Y*	76 (97.4%)
	N	2 (2.6%)
Neoadjuvant	*Y*	36 (46.1%)
	N	41 (52.6%)
	Not Known	1 (1.3%)
Adjuvant Endocrine	*Y*	40 (51.3%)
	N	38 (48.7%)

Results: ctDNA detection levels



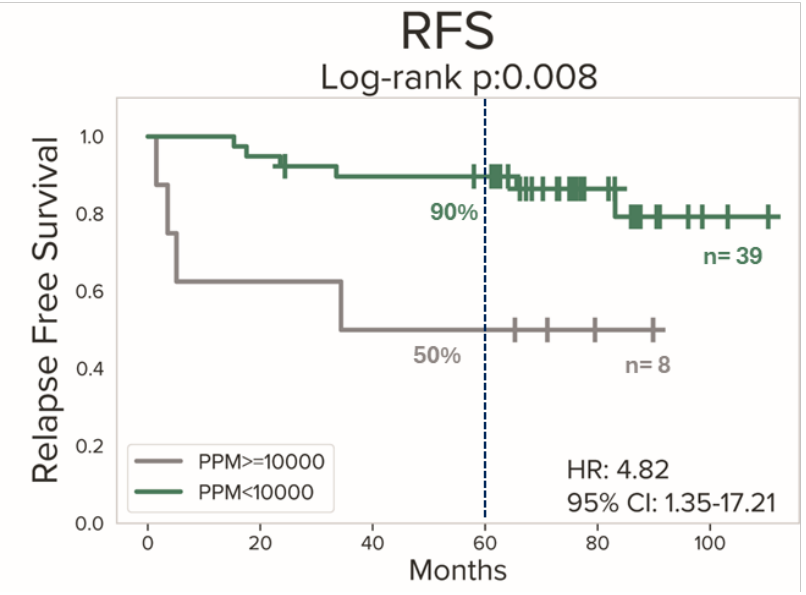
39% (45/115) of detections were in the ultrasensitive range below 100 PPM (below ~ 0.01% TF)

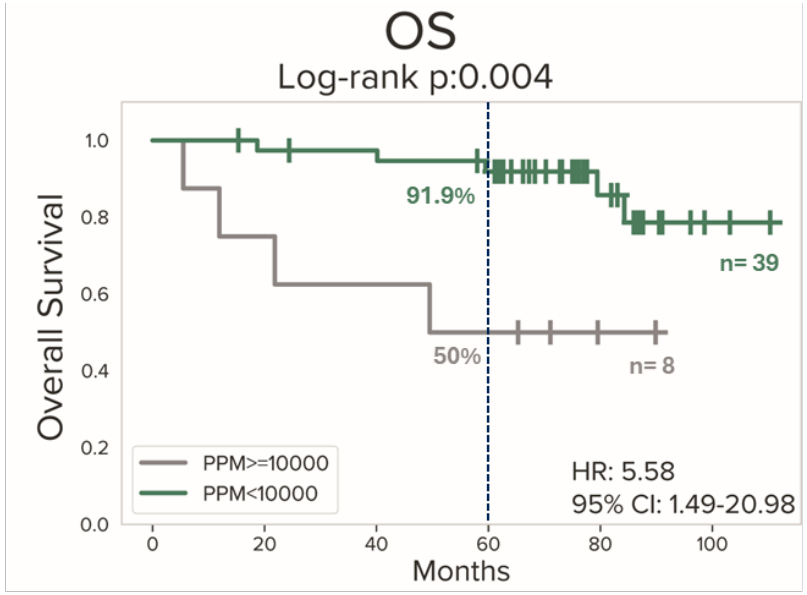
45% (18/40) of post-surgery detections were in the ultrasensitive range

Median detection was **366 PPM** (range 3.73-112,011)

Median ctDNA level at first molecular relapse detection was **13.1 PPM** (range 2.28-14,204)

Results: Baseline detection at diagnosis prior to treatment

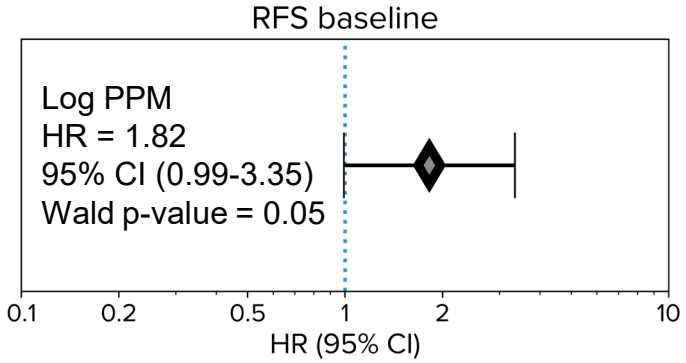


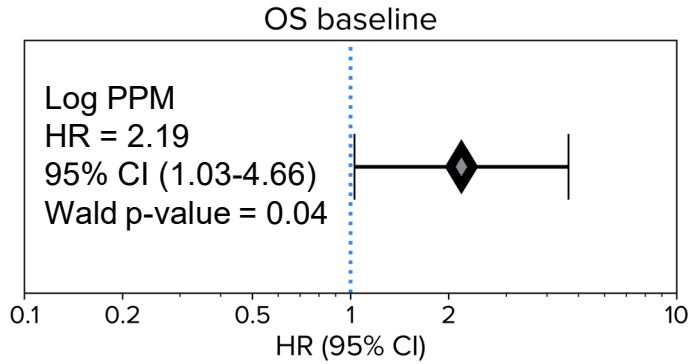


64% (50/78) patients had a baseline sample

98% (49/50) of baseline samples had ctDNA detected

(median 1,225 PPM; range 3.73-112,011.1 PPM)

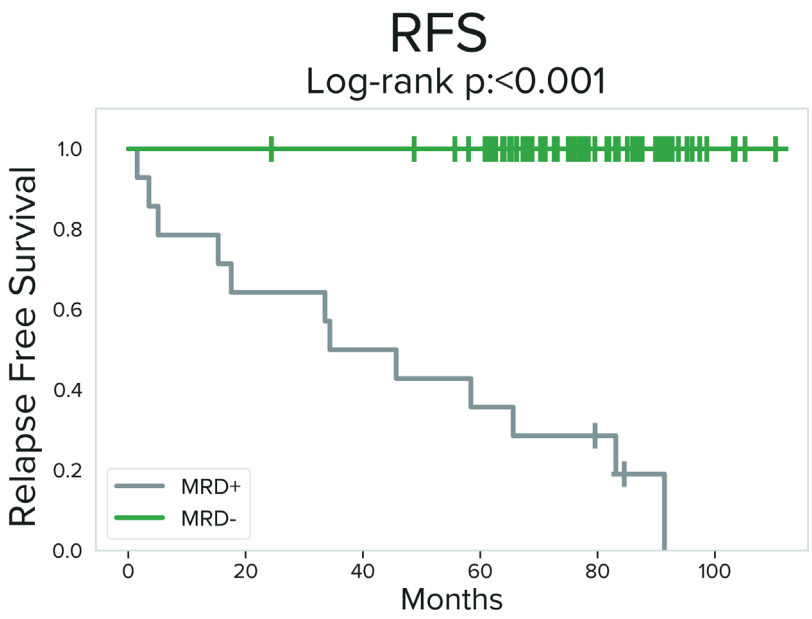




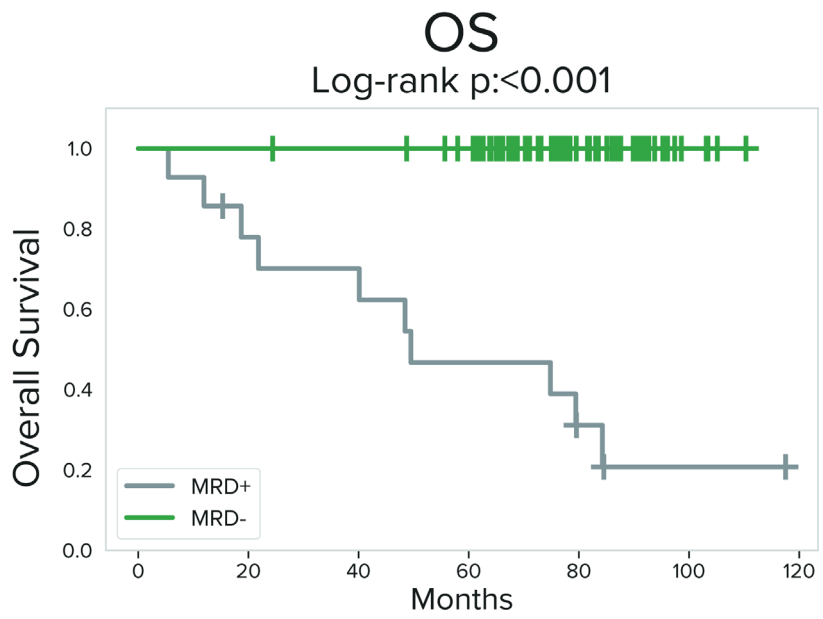
Continuous hazard models of log-transformed baseline PPM values were prognostic of increased RFS **(HR=1.82)** and OS **(HR=2.19)**

* A single ER+HER2- patient had undetectable ctDNA at diagnosis

Results: Molecular Relapse detection



MRD+						
At risk	14	9	7	5	3	0
Censored	0	0	0	0	1	2
Events	0	5	7	9	10	12
MRD-						
At risk	60	60	59	56	25	4
Censored	0	0	1	4	35	56
Events	0	0	0	0	0	0



MRD+							
At risk	14	10	9	6	3	1	0
Censored	0	1	1	1	2	3	4
Events	0	3	4	7	9	10	10
MRD-							
At risk	60	60	59	56	25	4	0
Censored	0	0	1	4	35	56	60
Events	0	0	0	0	0	0	0

Median Lead Time **15** months (range 4-41 months) over clinical relapse

Median Overall Survival

ctDNA detected **62** months
ctDNA undetected – NR

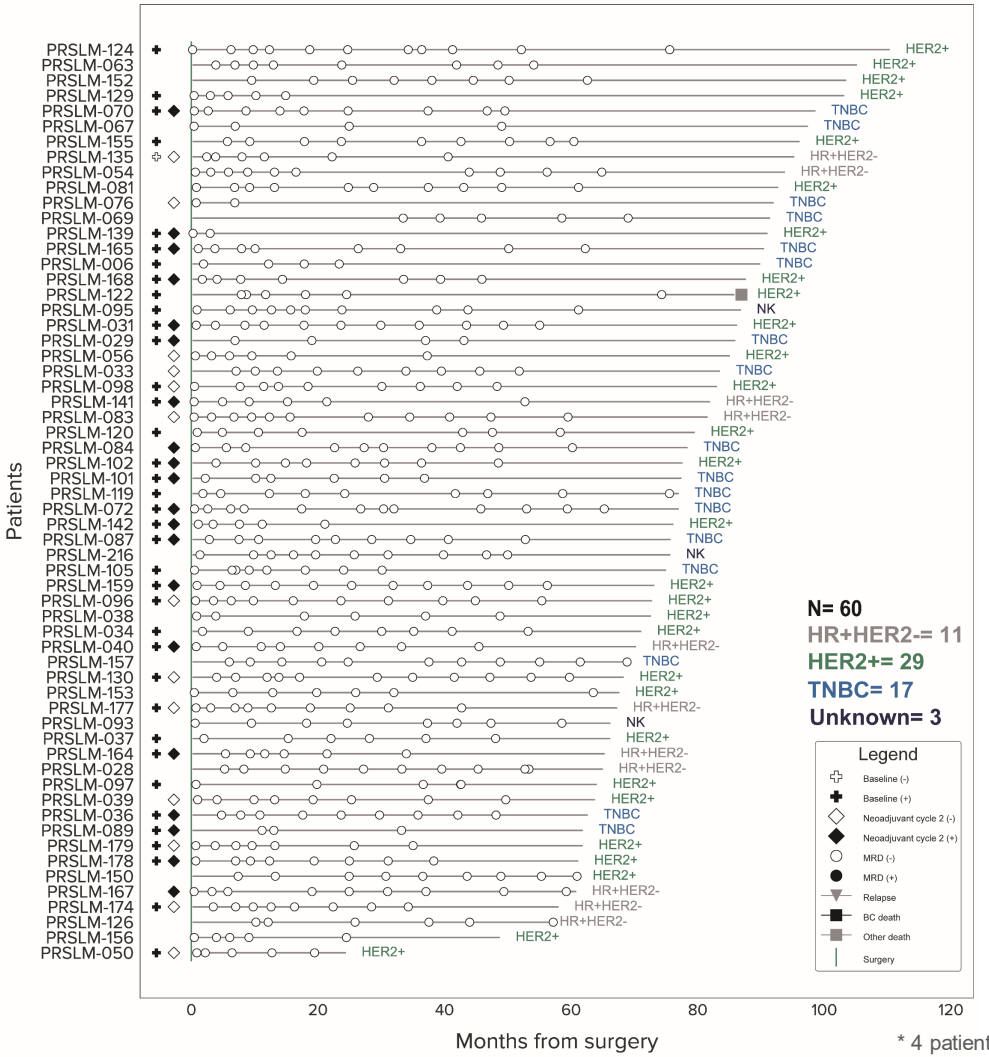
Longitudinal performance*:
Sens= 100%; spec = 100%;
PPV = 100%; NPV = 100%

*3 patients had MRD detected post-surgery but subsequently cleared in all remaining timepoints

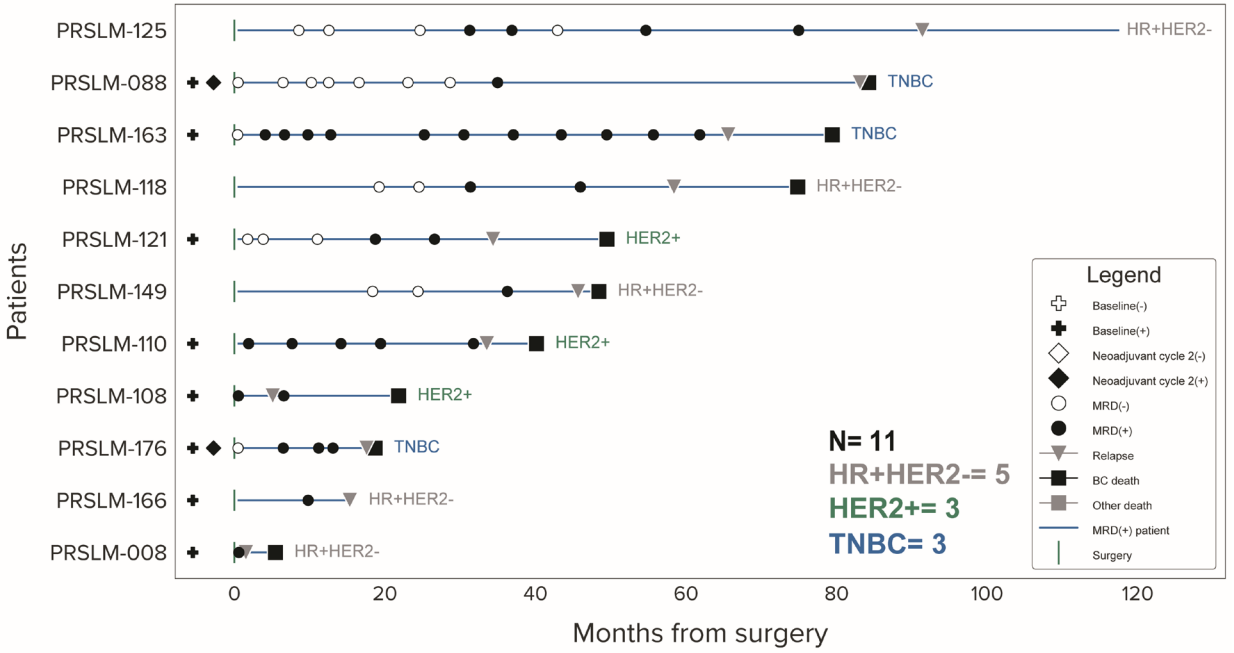
PRESENTED BY: Isaac Garcia-Murillas, PhD

Results: MRD tracking

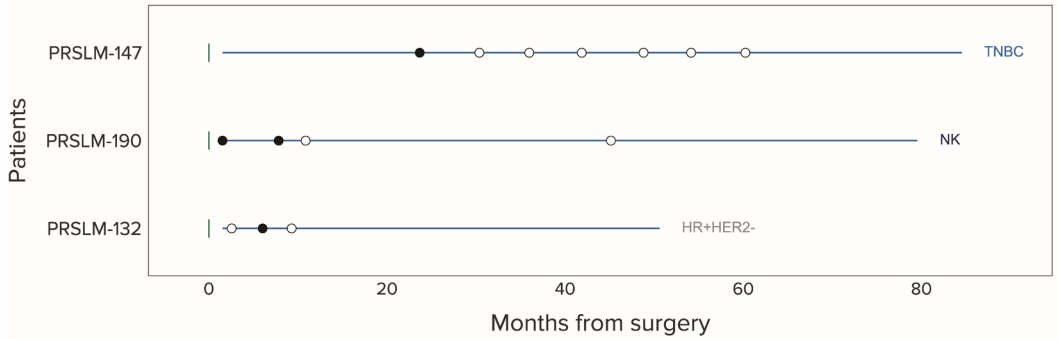
77% ctDNA non detected post-surgery= no relapse



14% ctDNA detected post-surgery= relapse



4% ctDNA detected post-surgery or follow-up and no relapse

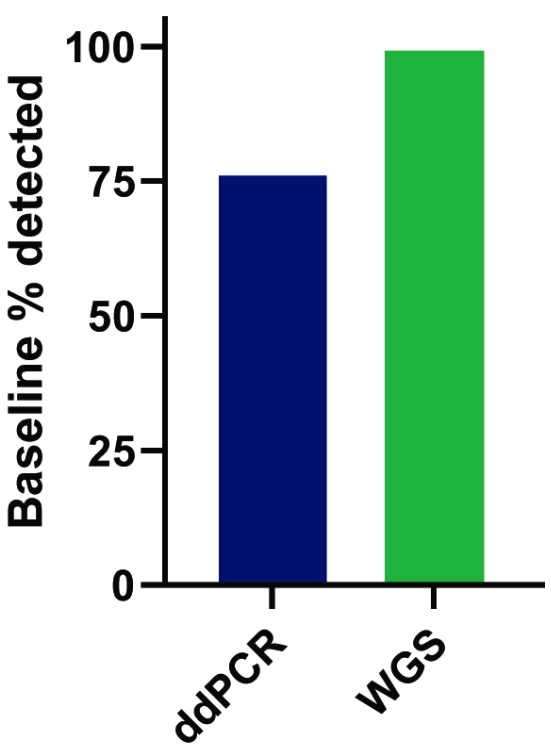


* 4 patients (5%) had no samples post-surgery or during follow-up

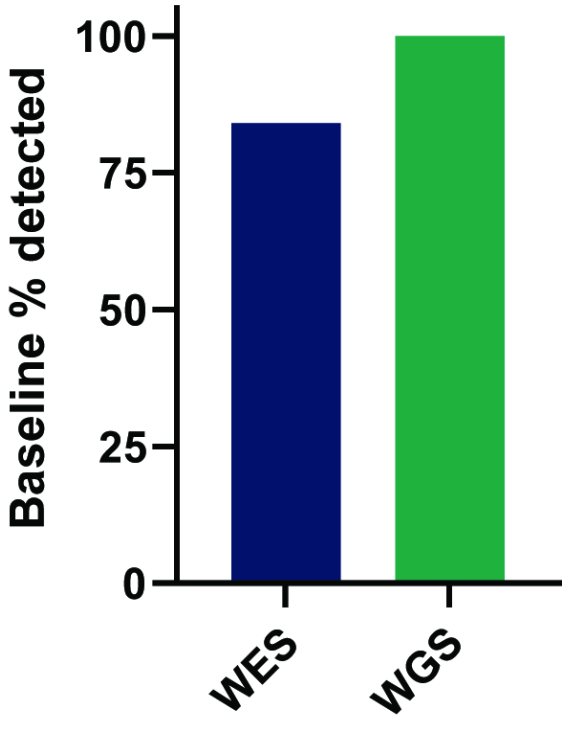
PRESENTED BY: Isaac Garcia-Murillas, PhD

Results: Assay Comparison in the same patient

Baseline detection

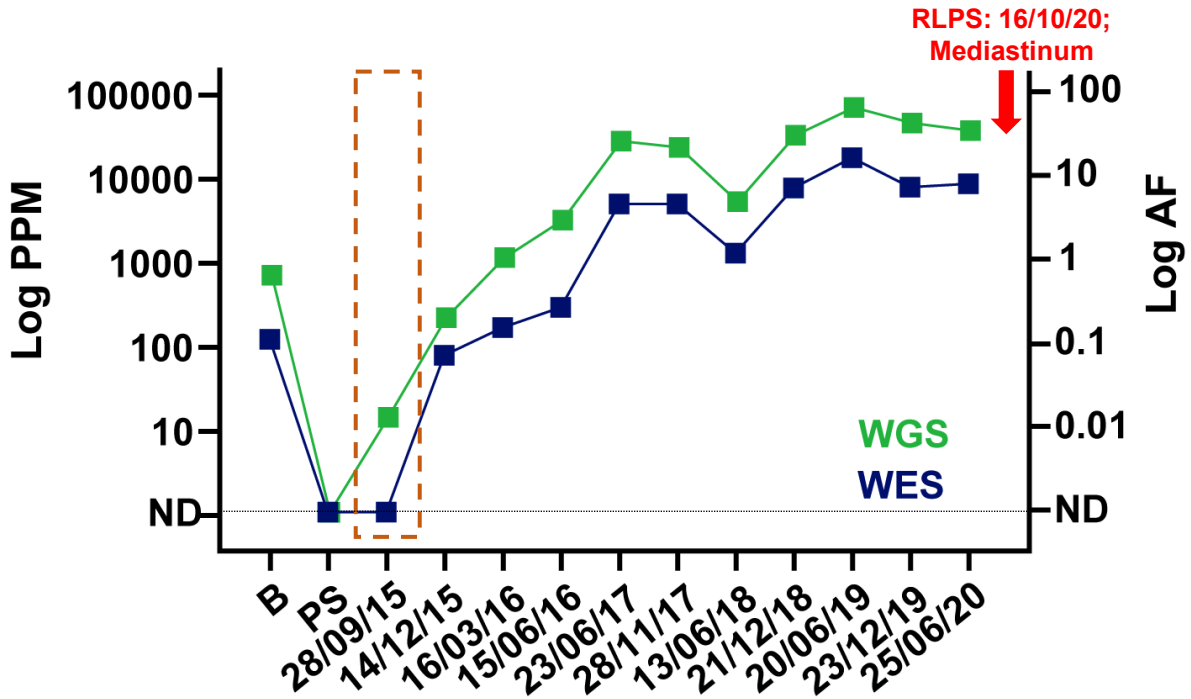


Baseline detection **n=17**
ddPCR= 76% (13/17)
WGS= 100% (17/17)



Baseline detection **n=19**
Exome= 84% (16/19)
WGS= 100% (19/19)

Example Improved lead time



IDC; TNBC; NACT, No Adjuvant Tx; 70 months follow-up

3 months lead time over WES powered approach

Conclusions

- Ultrasensitive detection with a bespoke whole genome sequencing based tracking assay improves detection of ctDNA at baseline and during follow-up and increases lead time over clinical relapse

- ctDNA detection during follow-up strongly associates with worse relapse free and overall survival

- An ultrasensitive detection assay identifies early MRD+ patients that clear ctDNA and do not relapse during long follow-up, with the biology of this currently not understood

Acknowledgements

All Patients and their families in the studies

All investigators, research coordinators, and site staff

Molecular Oncology Lab at the Breast Cancer Now Toby Robins Research Centre at The Institute of Cancer Research and The Ralph Lauren Centre for Breast Cancer Research at the Royal Marsden Hospital

Aya Amenssag
Iseult Browne
Niamh Cunningham
Jasmine Florence
Samira Futa
Andriani Hadjiconstanti
Maria Herrera Abreu
Margaret Hills
Sarah Hrebien
Uzma Khalid
Istvan Kleijn
Callum Loader
Elena Lopez Knowles
Sarah Mearns
Laura Morrison
Emma O'Neill
Nida Pasha
Alex Pearson
Ruth Sardinha
Gene Schuster
Heena Shah
Lixuan Sim
Prithika Sritharan
Claire Swift

ICR The Institute of Cancer Research

BREAST CANCER NOW The research & care charity

The Royal Marsden NHS Foundation Trust

The ROYAL MARSDEN Cancer Charity

NHS National Institute for Health Research

PRESENTED BY: Isaac Garcia-Murillas, PhD

Lay Summary

- Detection of Minimal Residual Disease (MRD) (minutes amount of cancer left after initial therapy), with tumor-derived DNA in the blood, a liquid biopsy, allows the identification of patients at risk of relapse and offers the potential to tailor treatments for primary breast cancer patients

- Current approaches can identify patients, on average, up to 11 months before they relapse clinically

- Here we present an ultrasensitive method that extends the lead time over clinical relapse to an average of 15 months, and in some cases to over 41 months, improving on current approaches

PRESENTED BY: Isaac Garcia-Murillas, PhD