San Antonio Breast Cancer Symposium – December 9-12, 2025 #PS2-07-05

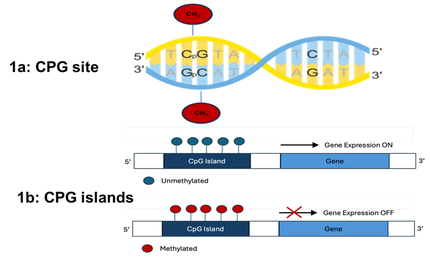
Methylation-based ctDNA Dynamics as a Biomarker for Treatment Response and Prognosis in Patients on the plasmaMATCH trial

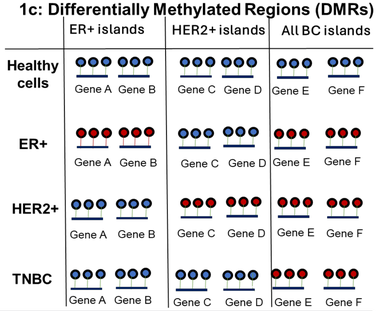
Iseult M. Browne[1,2], Rosalind J. Cutts[3], Aya Amenssag[3], Claire S. Swift [2], Callum Loader [2], Sarah Hrebien[3], Monica Esteban-Garcia[1,2], Javier Pascual[1], Lucy S. Kilburn[4], Alex Pearson[3], Andrew M. Wardley[5], Iain R. Macpherson[6], Richard D. Baird[7], Rebecca Roylance[8,9], Isaac Garcia-Murillas[2,3], Judith M. Bliss[4], Alistair Ring[1] and Nicholas C. Turner [1,2,3].

1. Breast Unit, The Royal Marsden NHS Foundation Trust, London, UK; **2.** The Ralph Lauren Centre for Breast Cancer Research, The Royal Marsden Hospital NHS Foundation Trust, London, UK; **3.** The Breast Cancer Now Toby Robins Research Centre, The Institute of Cancer Research, London, UK; **4.** ICR-CTSU, The Institute of Cancer Research, London SM2 5NG, UK; **5.** The Christie NHS Foundation Trust, Manchester, UK; **6.** School of Cancer Sciences, University of Glasgow, Glasgow, UK; **7.** Cancer Research UK Cambridge Centre, Cambridge, UK; **8.** University College London Hospitals NHS Foundation Trust, London, UK; **9.** NIHR University College London Hospitals Biomedical Research Centre, London, UK.

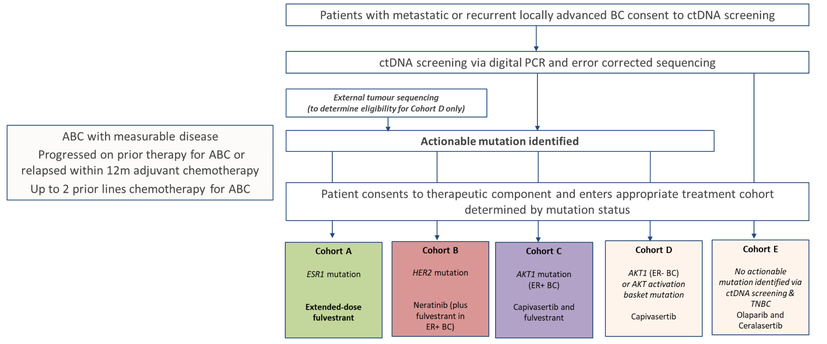
BACKGROUND

- Early assessment of circulating tumour DNA (ctDNA) dynamic changes on treatment predict response to therapy.
- Previous studies have tracked mutations in plasma, which requires prior knowledge of the tumour mutations and is at risk of false results tracking mutations in clonal haematopoiesis.
- DNA methylation changes **occur early** in cancer development, are more **abundant** than mutations, and are highly tissue and **cell type specific**.
- In humans, methylation occurs at **CpG sites,** and individual methylation sites are clustered together to form **CpG islands**.
- DNA methylation is an important regulator of breast cancer (BC), which displays distinct differentially methylated regions (**DMRs**) between subtypes. We have developed a robust **tumor agnostic** ctDNA assay – **Breast Cancer Methylation Assay (BCMA) -** that tracks the level of aberrant breast cancer specific DNA methylation in plasma.
- We assessed the potential of early on-treatment changes in ctDNA breast cancer methylation levels, to predict progression-free survival (PFS) and objective response rate (ORR) to therapy.





PlasmaMATCH Trial Design and Treatment



METHODS

- Patients enrolled on the plasmaMATCH trial with matched cycle 1 day 1 (**C1D1**) and cycle 2 day 1 (**C2D1**) plasma samples available for analysis were included
- **Cohorts A-D** were analyzed together (ER+ BC on targeted therapy), and **cohort E** was analyzed separately (TNBC on Olaparib (PARP) and Ceralasertib (ATR) inhibitor combination).
- An in-house capture based enzymatic ctDNA-based methylation assay **(BCMA)** targeting **1,297 DMRs** was used to distinguish the presence of breast cancer and level of ctDNA.
- Libraries were sequenced on Illumina platforms (NextSeq or NovaSeq) to a target of **300X**
- Tumor ctDNA levels were inferred from **Methylation Fraction (MF)** which is estimated by summing methylated reads/total reads for the top 60 regions based on high entropy/high subtype methylation in the methylation panel
- **Circulating methylation ratio (CMR)** is calculated as the ratio of C2D1 MF/ C1D1 MF.
- We evaluated the association of **baseline MF**, and **median change in CMR at C2D1**, with PFS and ORR.

Twist Biosciences NEBNext Enzymatic Methyl-seq Library Preparation Protocol and Fast Hybridization Target Enrichment

Extraction	Quantification & Electrophoresis	End Repair / dA-Tailing	Em-Seq Adaptor Ligation	Oxidation of 5mC and 5hmC	Deamination of C to U	PCR Amplification	Sequencing
On QIASymphony	Qubit & Tapestation. Input required ≥14ng	DNA is end-repaired and dA-tailed	DNA is ligated to the EM-Seq adaptors	TET2 and Oxidation Enhancer protect 5mC/5hmC from deamination	APOBEC deaminates C to U; oxidised forms of 5mC/5hmC are not deaminated	Library amplification using NEBNext Q5U Master Mix and index primers	Sequencing on Illumina platform

RESULTS

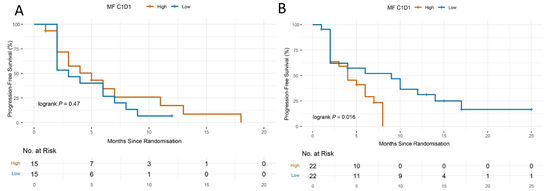
Baseline Characteristics

	Cohorts A-D All evaluable patients N=30		Cohort E All evaluable patients N=44	
	n	%	n	%
Age group (years)				
<50	7	23	14	32
50-59	9	30	15	34
60-69	10	33	11	25
≥70	4	13	5	11
Histological type				
Ductal	19	63	42	95
Lobular	6	20	1	2.3
Mixed ductal & lobular	1	3.3	0	0
Not known/Missing	4	13	1	2.3
Tumour grade				
G1	2	6.7	0	0
G2	19	63	6	14
G3	6	20	38	86
Not known/Missing	3	10	0	0
Molecular subtype				
ER+, HER2-	22	73	0	0
ER+, HER2+	2	6.7	0	0
ER-, HER2+	1	3.3	0	0
TNBC	0	0	44	100
ER Unknown, HER2-	1	3.3	0	0
ER+, HER2 unknown	4	13	0	0
Disease sites				
Visceral	28	93	36	82
Soft tissue/nodal	16	53	35	80
Bone	27	90	13	30
Total lines of systemic therapy				
0	0	0	14	32
1	6	20	20	45
2	7	23	9	20
3	7	23	1	2.3
>3	10	33	0	0

Baseline MF

- Median baseline MF for cohorts A-D was 0.121 and cohort E was 0.042

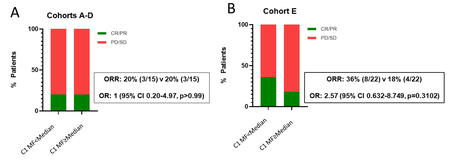
mPFS split by median baseline MF (A) Cohorts A-D, (B) Cohort E



mPFS 3 (low) v 5 (high)
HR = 1.16; 95% CI: 0.81-1.60; p=0.47

mPFS 9 (low) v 4 (high)
HR = 0.68; 95% CI: 0.49-0.94; p=0.023

Association of baseline MF with ORR (A) Cohorts A-D, (B) Cohort E

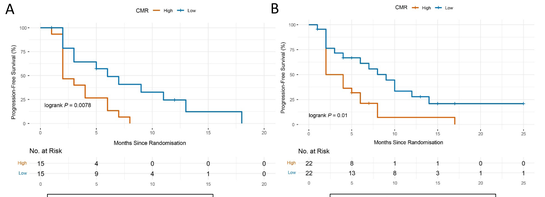


RESULTS

MF dynamics C2D1

- Median CMR at C2D1 for cohorts A-D was 0.656 and cohort E was 0.428

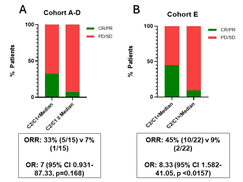
mPFS split by median CMR C2D1 (A) Cohorts A-D, (B) Cohort E



mPFS 6 (low) v 2 (high)
HR = 0.66; 95% CI: 0.49-0.88; p=0.001

mPFS 9 (low) v 3 (high)
HR = 0.52; 95% CI: 0.26-0.99; p=0.016

Association of CMR with ORR (A) Cohorts A-D, (B) Cohort E



ORR: 33% (5/15) v 7% (1/15)
OR: 7 (95% CI 0.931-87.33, p=0.168)

ORR: 45% (10/22) v 9% (2/22)
OR: 8.33 (95% CI 1.582-41.05, p <0.0157)

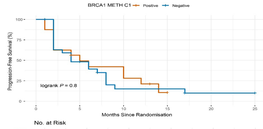
High baseline MF was associated with shorter PFS (4 v 9 months) and lower ORR (18 v 36%) for patients in Cohort E

There was no association with baseline MF and outcomes for Cohorts A-D, though this may be due to small sample size and heterogenous population / treatments received

In all Cohorts (A-D and E) splitting patients by median CMR dynamics was predictive of PFS and ORR

BRCA methylation and outcomes

mPFS split by BRCA1 methylation at baseline Cohort E



mPFS 5 (BRCA1Meth+) v 4 (BRCA1Meth-)
HR = 0.79; 95% CI: 0.45-1.89; p=0.814

CONCLUSIONS AND NEXT STEPS

- Using a tumour agnostic breast cancer ctDNA methylation assay **(BCMA)**, we show that both baseline ctDNA methylation levels, and on treatment ctDNA methylation dynamics identify patients who will respond to targeted therapy.
- There was no association between *BRCA1* methylation and outcomes, suggesting the sensitivity to PARP and ATR inhibition combination of cohort E was not mediated by *BRCA1* methylation.
- Subject to further independent validation, ctDNA methylation dynamics may present a simple way to identify patients who are responding to novel therapy combinations early.

ACKNOWLEDGEMENTS

plasmaMATCH is funded by Cancer Research UK (CRUK/15/010, C30746/A19505) with additional support from AstraZeneca, Puma Biotechnology, Guardant Health, and BioRad. Grateful thanks to all trial participants and their families. We thank Breast Cancer Now for funding this work as part of Programme Funding to The Toby Robins Breast Cancer Research Centre, The Institute of Cancer Research, London. Thanks also to staff at participating centres, the ICR-CTSU trial team, the staff at central laboratories, and Syed Haider and his team in the Breast Cancer Now Toby Robins Research Centre Bioinformatics Core Facility for bioinformatics support. plasmaMATCH is co-sponsored by the Institute of Cancer Research and the Royal Marsden National Health Service Foundation Trust. ICR-CTSU is supported by the Cancer Research UK core grant (C1491/A25351). plasmaMATCH is supported by the National Institute for Health Research (NIHR) Manchester Clinical Research Facility at the Christie Hospital, Manchester, UK, and by the Cancer Research UK Cambridge Centre, the Cambridge NIHR Biomedical Research Centre, and the Cambridge Experimental Cancer Medicine Centre, Cambridge, UK. plasmaMATCH is supported at participating sites in England by the NIHR Clinical Research Network, in Scotland by the Chief Scientist Office, and in Wales by Health and Care Research Wales. This study represents independent research supported by the NIHR Biomedical Research Centre at the Royal Marsden National Health Service Foundation Trust and the Institute of Cancer Research, London, UK. The views expressed are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care. The authors also acknowledge past and present colleagues on the plasmaMATCH trial management group, the independent data monitoring committee, and the trial steering committee, who provided oversight of the trial.

This presentation is the intellectual property of the author/presenter. Contact them at plasmamatch-icrctsu@icr.ac.uk for permission to reprint and/or distribute.