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Detecting subtype switching in metastatic breast cancer with circulating tumor DNA methylation profiling

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Introduction

Subtype switching in metastatic breast cancer (MBC), is a well described phenomenon.

Histological switches are associated with changes in tumor phenotype and aggressive clinical features which can be a major source of treatment failure if not identified.

We investigated whether subtype switching could be detected in liquid biopsies using a set of serial MBC samples analysed with the liquid biopsy-based *Breast Circulating Methylation Assay (BCMA)* [1].

Materials and Methods

The *BCMA* was developed to non-invasively detect breast cancer subtypes through identification of subtype-specific CpG methylation patterns on circulating tumor DNA. The assay is based on Differentiated Methylated Regions and has been validated on plasma samples from **65** healthy donors, and **269** samples from **150** breast cancer patients using a 10-fold stratified nested cross-validation with machine learning classifiers[1].

BCMA Training Cohort	Samples	Patients	Age
ER+HER2-	183	95	61 (31-94)
ER-PR+	4	1	55 (53-56)
ER-HER2+	15	12	55.5 (28-73)
ER+HER2+	24	13	48 (40-58)
TNBC	43	30	56 (35-85)
All BC	269	150	58 (28-94)
HD	65	63	36 (18-65)

Plasma samples from patients enrolled in five trials at the Royal Marsden Hospital, predominately: **ABC-Bio** and **PlasmaDNA**, were analyzed.

Four independent classifiers were developed to distinguish breast cancer subtypes from plasma DNA, and to identify the presence of breast cancer.

Classifier	Sample counts (+/-)	AUC-ROC	Median sensitivity	Median specificity
BC vs healthy	269/65	0.988	96.3%	100%
ER+ vs ER-	183/62	0.948	92.7%	100%
HER2+ vs HER2-	39/230	0.853	75%	95.7%
TNBC + vs TNBC-	43/226	0.932	87.5%	95.7%

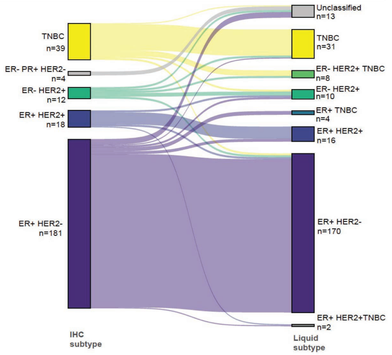
Results

BCMA Performance

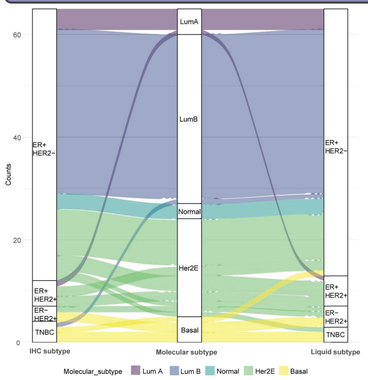
In the cohort 254/269 samples had breast cancer detected by the BCMA assay (95%) at 100% specificity.

Liquid Classifier results were assessed by comparison with tissue subtypes using standard IHC criteria.

Only 13 (5%) samples failed to receive a BCMA classification, 14 (5.5%) samples received diverging classifications.



Molecular Subtype



Molecular subtypes were generated on **65** tissue biopsies and compared against BCMA subtype.

Of the **57** samples that had an IHC tissue HER2- (negative) subtype, **14** were HER2 enriched with **29%** (4/14) classified as HER2+ BCMA subtype.

Of the **43** samples that were not HER2 enriched (luminal A, luminal B, basal, normal), none were classified as a HER2+ BCMA subtype (*p* value= 0.0025).

Switcher Cohort

Switcher Cohort	
Number of samples	191
Number of patients	86
Median age (range)	59 (34-84)
Median Months between first and repeat biopsy (range)	63 (20-203)

Patients in this series had at least two tissue biopsies, with a first and repeat tissue biopsy used to detect subtype switching.

Subtype Switch

Subtype switch occurred in **19%** (37/191) of samples (17/86 patients). The BCMA correctly identified the subtype switch in **89.2%** (33/37) of these samples.

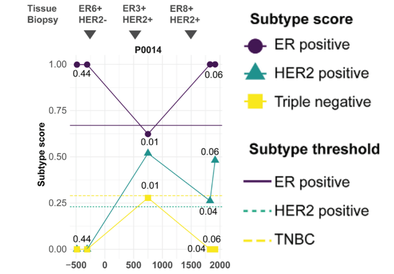
In **8%** (3/37) of samples both the original and the new subtype were co-detected with BCMA, suggesting both subtypes may co-exist.

Patients ER+HER2- on both tissue biopsies, **96%** (129/134) were BCMA ER+HER2- and **0%** (0/134) were BCMA TNBC.

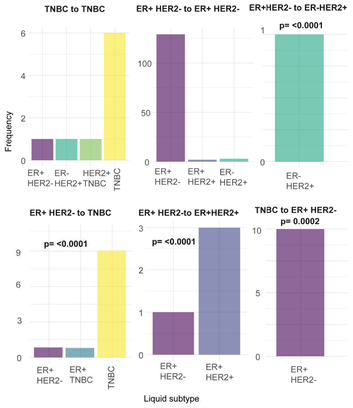
BC ER+HER2- on initial biopsy and TNBC on later biopsy, **9%** (1/11) were BCMA ER+HER2-, and **91%** (10/11) were BCMA TNBC. One sample was mixed ER+/TNBC by BCMA ($p<0.0001$).

BC TNBC on both tissue biopsies, **88%** (8/9) were BCMA TNBC and **11%** (1/9) were BCMA ER+HER2-.

BC TNBC on initial biopsy and ER+HER2- on later biopsy, **0%** were BCMA TNBC and **100%** (10/10) were BCMA ER+HER2- ($p=0.0002$).



BCMA results for patient showing acquired HER2 positivity, sample methylation fraction shown labelling timepoints.



Conclusions

Analysis of circulating tumour DNA with *Breast Circulating Methylation Assay (BCMA)* accurately identifies tumors that switch subtype using a non-invasive approach. In MBC multiple subtypes may co-exist in the same tumor/patient. Clinical trials are warranted to assess whether BCMA subtyping has the potential to direct MBC treatment.

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References: 1. Nida Pasha et al. Discriminating subtypes in advanced breast cancer with ctDNA methylation profiling. JCO 42, 1013-1013(2024).