Agreement of cell-free DNA methylation-based molecular breast subtyping and tissue subtyping in hormone receptor positive metastatic breast cancer: a clinical cohort analysis

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BACKGROUND

- Hormone receptor (HR) and HER2 status, typically assessed on tissue biopsies, are essential for breast cancer (BC) management.
- As multiple therapeutic options are available for HR+ metastatic BC (mBC), ongoing subtype assessment may be recommended to monitor changes under the selective pressure and/or evolution of disease.
- Cell-free DNA (cfDNA) showed reliability for biomarker evaluation and high concordance with tissue-based methods and may be an opportunity for non-invasive, *real -time* molecular subtyping assessment.

This study aimed to evaluate agreement between cfDNA methylation-based molecular breast subtyping (MBS) and tissue status in a clinical cohort who underwent cfDNA testing as part of routine clinical care.

METHODS

- Blood samples drawn for circulating tumor DNA (ctDNA) clinical testing (Guardant360®) from mBC patients with HR+ disease on the most recent tissue biopsy at Weill Cornell Medicine from Nov 2024-June 2025 were queried.
- A novel cfDNA MBS signature was applied to test results through bioinformatics and/or residual sample testing. MBS confidence scores for HR+/HER2+ where tumor fraction (TF) >0.5% were applied[1]. In this research setting, MBS predicted overall subtype was generated to compare it to the most recent tissue biopsy available.
- Clinical and genomic factors were annotated for correlation; Fisher's exact and t-tests were used for statistical analysis

RESULT

Figure 1. Patient consort diagram.
Of 73 plasma sample collected, 42 were evaluable for MBS. *Of those not evaluable, 16/31 (51.6%) had maximum variant allele frequency <1% and two had no circulating tumor DNA detected. Notably, 24/31 (77%) non-evaluable samples had >1 genomic alteration annotated as clonal hematopoiesis.

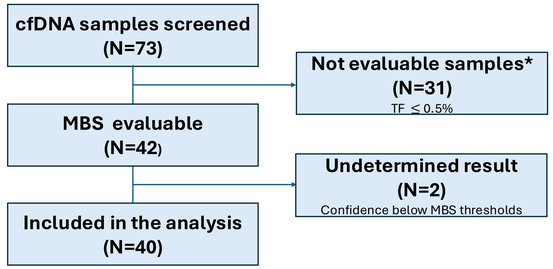


Table 1. Agreement between predictive subtyping by MBS (blood) and histology (tissue).
Data comparing tissue subtyping to MBS show high agreement based on the predominant subtype identified by MBS, with varying confidence scores by caller. The median confidence score for HR+ prediction was 80% (range 30–90%). Disagreement (n = 9) was mainly driven by HER2 classification.

	HR+/HER2- (Blood)	HR+/HER2+ (Blood)	HR-/HER2+ (Blood)
HR+/HER2- (Tissue)	27	3	0
HR+/HER2+ (Tissue)	5	4	1

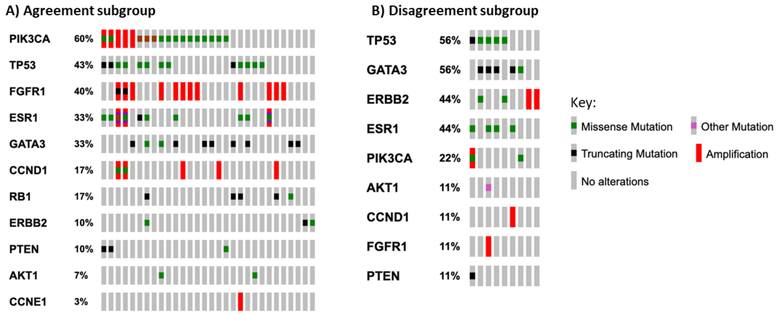
MBS demonstrated a positive percent agreement of 77.5% (N=31) with tissue subtyping (Fisher's exact test p = 0.003).

Table 2. Baseline clinical and molecular characteristics of the evaluable samples cohort.
Overall, no clinicopathological factors were significantly associated with MBS/tissue agreement, except for tissue subtypes (Table 1) and HER2 low expression.

Characteristic	Overall N = 40[1]	Agree N = 31[1]	Disagree N = 9[1]	p-value[2]
HER2 Status (Tissue)				**0.003**
HER2 0	10 (25%)	8 (26%)	2 (22%)	
HER2 low (1/2+)	20 (50%)	19 (61%)	1 (11%)	
HER2 3+/2+ FISH amplified	10 (25%)	4 (13%)	6 (67%)	
Site of Tissue Biopsy				0.28
Bone	9 (23%)	8 (26%)	1 (11%)	
Breast	10 (25%)	7 (23%)	3 (33%)	
Liver	15 (38%)	11 (35%)	4 (44%)	
Lymph node	5 (13%)	5 (16%)	0 (0%)	
Skin/soft tissue	1 (2.5%)	0 (0%)	1 (11%)	
Number of Metastatic Sites	3.0 (2.0, 3.5)	3.0 (2.0, 4.0)	3.00 (2.00, 3.00)	0.81
Number of Prior therapies	2.5 (0.0, 4.0)	2.00 (0.0, 4.0)	3.00 (3.0, 4.00)	0.42
Prior CDK4/6 Therapy	27 (68%)	20 (65%)	7 (78%)	0.69
Tumor Fraction	7.96 (1.58, 44.79)	8.06 (1.78, 47.45)	5.26 (0.82, 21.35)	0.30
Time from tissue biopsy to liquid biopsy (days)	342 (68, 722)	354 (78, 833)	300 (59, 448)	0.65

[1]n (%); Median (Q1, Q3)
[2]Fisher's exact test; Wilcoxon rank sum test

Figure 2. Genomic alteration differences in genes of interest stratified by MBS agreement groups.
When assessing alterations in breast cancer genes of interest, no differences were found between agreement and disagreement subgroups.



CONCLUSION

- This is the first *real-world* study to evaluate the performance of a novel blood-based MBS in a clinical cohort.
- cfDNA MBS showed strong agreement with tissue subtyping and reproduced prior accuracy, independent of clinicopathological, therapeutic, or genomic factors.
- Subtype switching, tumor heterogeneity, and/or lower MBS confidence may explain disagreement with tissue results.
- Blood-based MBS represents a feasible tool to complement standard tissue-based subtyping, enabling *real-time* assessment of disease heterogeneity and evolution.
- Prospective studies are warranted to further define its clinical utility, impact on patient management, and complementarity with actionable genomic profiling and matched targeted therapies.

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References:
1.Tolkunov D. et al.; Cancer Res (2025) 85 (8_Supplement_1): 3247.

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