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Detection of predictive mutations and HER2/neu expression from circulating tumor cells of metastatic breast cancer patients: new opportunities by analyzing high blood volumes obtained by diagnostic leukapheresis

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Background

Tumor-derived material obtained through liquid biopsy—most commonly circulating tumor DNA (ctDNA)—has become a key biomarker for guiding targeted therapy in metastatic breast cancer. However, ctDNA is not released by all tumors and does not provide information on protein expression. In contrast, circulating tumor cells (CTCs) are intact cells that allow for multiparametric analyses, including both genomic profiling and protein-level assessments such as HER2 expression.

Despite these advantages, the clinical utility of CTCs is often limited by their extremely low abundance. To enhance CTC yield, the diagnostic leukapheresis (DLA) offers a powerful alternative. By processing several liters of blood, DLA enables the density-based pre-enrichment of mononuclear cells (MNCs), including rare CTCs.

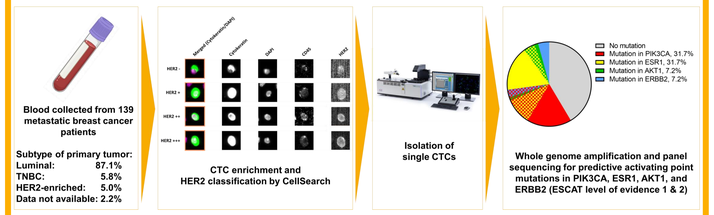
In this study, we compared the utility of CTCs with ctDNA and tissue biopsies to assess their respective and complementary roles as biomarkers in metastatic breast cancer.

Methods

To detect CTCs, 7.5 mL blood samples from 139 patients with metastatic breast cancer were analyzed using the CellSearch system, supplemented by immunofluorescence-based assessment of HER2 expression. Of these, 41 samples were retrospectively selected from a cohort of the DETECT-III clinical trial; 98 samples were collected prospectively.

Single CTCs were isolated via micromanipulation, followed by whole genome amplification of genomic DNA. Targeted sequencing was performed using a next generation sequencing (NGS) panel covering predictive hotspot mutations in PIK3CA, ESR1, AKT1, and ERBB2, and results were compared with matched tissue biopsies. In parallel, ctDNA isolated from matched plasma samples was analyzed by digital droplet PCR for hotspot mutations in PIK3CA and AKT1.

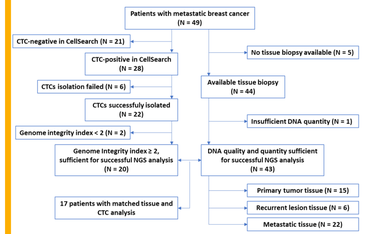
Additionally, from DLA products of nine patients, CTCs were enriched from samples adjusted to 2 × 10^9 MNCs using the flow enrichment target capture Halbach (FETCH) technology. Single CTCs were then isolated by fluorescence-activated cell sorting and analyzed by NGS.

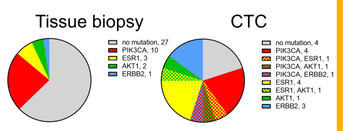


→ **Reliable analysis of HER2 expression and predictive mutations from CTCs**

Figure 1: Analysis of HER2 expression and predictive mutations from CTCs

CTCs compared to most recent tissue biopsies





	Predictive mutation	
	Tissue	CTCs
Patient 4	-	ESR1 (E380Q) / AKT1 (E17K)
Patient 7	PIK3CA (H1047R)	PIK3CA (H1047R)
Patient 8	-	PIK3CA (H1047R) / ERBB2 (S310F)
Patient 9	ERBB2 (V777L)	ERBB2 (V777L)
Patient 10	AKT1 (E17K)	PIK3CA (H1047R) / AKT1 (E17K)
Patient 12	-	-
Patient 13	PIK3CA (H1047R)	PIK3CA (H1047R)
Patient 15	PIK3CA (H1047L)	PIK3CA (H1047L)
Patient 16	-	ESR1 (Y537S)
Patient 17	-	-
Patient 20	-	ESR1 (D538G)
Patient 24	-	-
Patient 26	-	-
Patient 27	ESR1 (L536H)	ESR1 (Y537S)
Patient 28	PIK3CA (H1047R)	PIK3CA (H1047R) / ESR1 (Y537S)
Patient 29	PIK3CA (E545K)	PIK3CA (E545K)
Patient 32	-	-

- no mutation detected

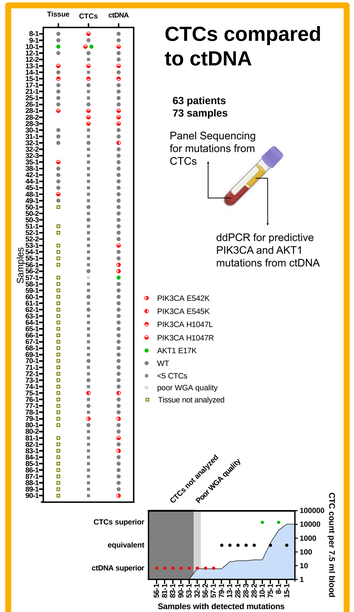
→ **≥18 CTCs: 100% sequencing success of single CTCs; 1–8 CTCs: success rate of 38.5%**

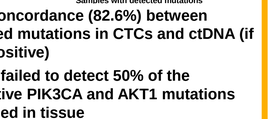
→ **In case CTC sequencing was successful: mutations identified in matched tissue biopsies were also detected in CTCs**

→ **Additional mutations identified in CTCs that were not detectable from tissue**

Figure 2: Compared mutation analysis of CTCs and tissue

CTCs compared to ctDNA





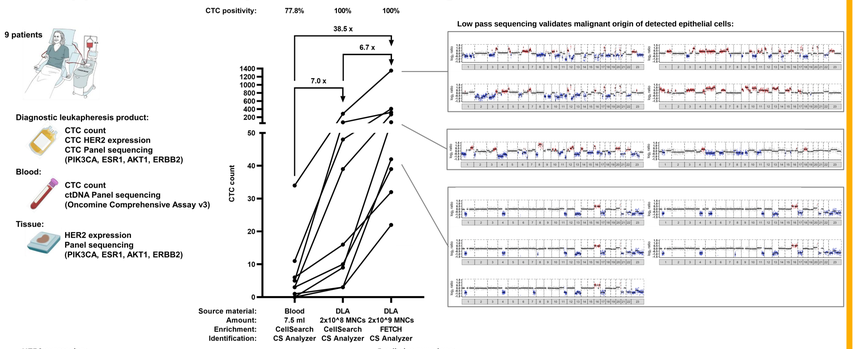
→ **High concordance (82.6%) between detected mutations in CTCs and ctDNA (if CTC-positive)**

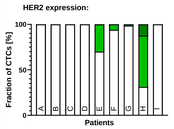
→ **ctDNA failed to detect 50% of the predictive PIK3CA and AKT1 mutations identified in tissue**

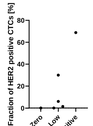
→ **Superior sensitivity of CTCs over ctDNA when ≥19 CTCs were available for analysis**

Figure 3: Compared mutation analysis of CTCs and ctDNA

New opportunities: Analysis of CTCs from high blood volumes







Predictive mutations:

	CTC	Time matched tissue biopsy	ctDNA
Patient A		insufficient material for molecular analysis	-
Patient B	PIK3CA E542K	analysis pending	-
Patient C	PIK3CA E545K	analysis pending	analysis pending
Patient D	PIK3CA E545K	no tissue biopsy performed	no plasma available
Patient E	PIK3CA E542K / ESR1 L539P	insufficient material for molecular analysis	PIK3CA E542K / ESR1 L539P
Patient F	PIK3CA E545K	Biopsy 1: - / Biopsy 2: PIK3CA E545K	-
Patient G	-	-	-
Patient H	-	-	-
Patient I	ESR1 Y537S	no tissue biopsy performed	ESR1 Y537S

- no mutation detected

→ **Application of DLA significantly increased CTC yield:**
- **Standard 7.5 mL blood samples yielded ≥19 CTCs in only 11.1% of patients (≥1 CTCs in 77.8%)**
- **DLA yielded ≥19 CTCs and resulted in successful sequencing in all 9 cases**

→ **HER2-positive CTCs: in 44.4% of the DLA samples**

→ **Predictive mutations: in 66.7% of the DLA samples**

→ **CTCs: Increased sensitivity compared to tissue or ctDNA**

Figure 4: Analysis of high blood volumes obtained by diagnostic leukapheresis

Conclusion

If present in sufficient numbers, CTCs enable more sensitive detection of predictive mutations than ctDNA and additionally provide HER2 protein expression data. DLA is critical to achieve the required CTC yield. CTC analysis from DLA products offers a valuable complement to tissue biopsies, particularly when metastases are inaccessible, biopsies require considerable effort, or are declined by patients.

LBCD Liquid Biopsy Center Düsseldorf

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