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A Novel Liquid Biopsy workflow to identify and characterize ER and HER2-positive CTCs in Metastatic Breast Cancer

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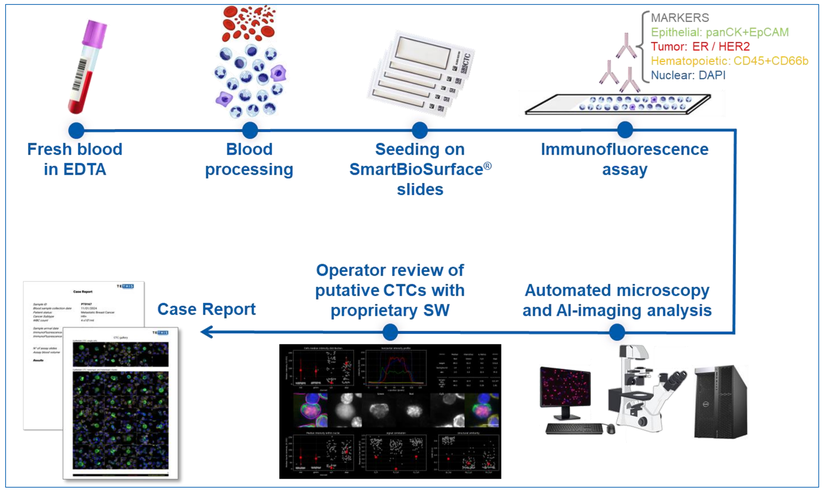
Background

- **Circulating Tumor Cells (CTCs) enumeration** can provide information on prognosis and treatment response [1];
- **Biomarkers characterization** of CTCs in routine practice is challenging [2];
- This study evaluates **a novel approach to address this limitation in Metastatic Breast Cancer** (MBC), aiming to provide the characterization of ER and HER2 protein expression in single cells.

Methods

- Blood samples in EDTA were collected from 28 Healthy Donors (HDs) among women in regular BC screening and 33 MBC patients (PTs) enrolled at San Raffaele Hospital (Milan, Italy), and processed within 6 hours of collection.
- After red blood cell lysis, the entire white blood cells (WBC) fraction was seeded on nanostructured titanium dioxide-coated slides (**SmartBioSurface® slides**) [3-5].
- CTCs were detected by an **immunofluorescence assay** targeting epithelial markers (pan-cytokeratins and EpCAM) and tumor-specific markers (ER or HER2), while WBCs were stained with a hematopoietic markers cocktail.
- **AI-driven detection** was performed at 10X magnification, followed by detailed imaging analysis at 40X magnification [6].
- **CTCs count** was normalized to the volume of analyzed blood and an exploratory cut-off of 0.3 CTCs/ml was used for the statistical analysis.

	HDs	MBC PTs
Number of participants	28	33
Age (years)		
median	38.5	53
range	23 – 65	33 – 80
WBCs count (10^6/ml)		
median	6.15	6.00
range	3.70 – 10.20	2.30 – 22.30
Histologic subtype		
Luminal A	–	5
Luminal B (ER+/Her2–)	–	13
Luminal B (ER+/Her2+)	–	2
HER2+	–	7
TNBC	–	6



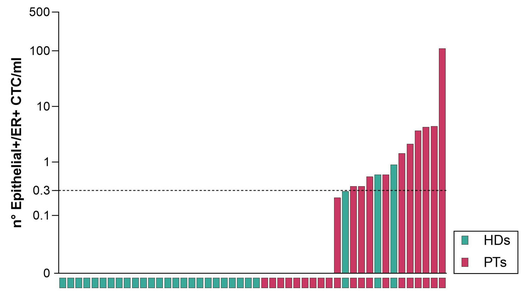
Liquid biopsy workflow using SmartBioSurface® slides: from blood to Case report
Blood samples in EDTA is processed within 6 hours for lysing red blood cells and isolating WBCs, to be seeded as a monolayer on SBS® slides at approximately 2.5 million WBCs/slide. After immunofluorescence staining, slides are scanned with an automated fluorescence microscope. CTCs are identified by AI-algorithm and their phenotype classification is attributed by trained operators using a proprietary software. For each sample, a Case Report is finally generated, detailing findings such as CTCs count and biomarkers characterization.

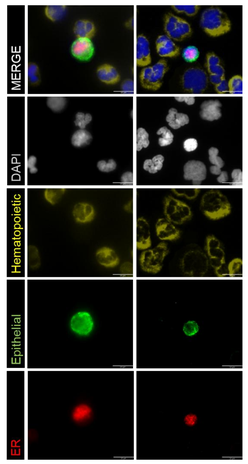
Results

1. **Epithelial+/ER+ CTCs** were successfully characterized in 10 of 20 (50.0%) MBC PTs classified as ER+ by histology, distinguishing them from HDs.
2. **Epithelial+/HER2+ CTCs** were found in 4 of 9 (44.4%) MBC PTs reported as HER2+ by histology, with a significant difference from HDs.
3. **Epithelial+ CTCs**, without tumor marker expression, were sufficient to discriminate overall ER+ and/or HER2+ PTs from HDs, but not TNBC PTs.
4. **CTC clusters** were identified and characterized in 6 MBC PTs (18.2%).

1. Characterization of Epithelial+/ER+ CTCs

	n° (%) with CTC/ml < 0.3	n° (%) with CTC/ml ≥ 0.3	Total n°	P value
HDs	26 (92.9%)	2 (7.1%)	28	0.0015
ER+ PTs	10 (50.0%)	10 (50.0%)	20	

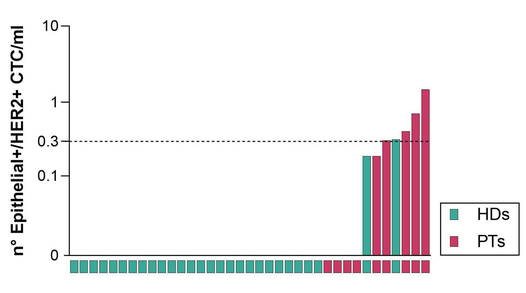


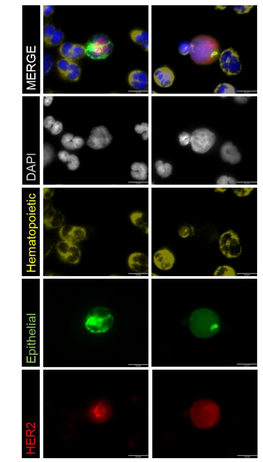


HDs and ER+ PTs were discriminated for Epithelial+/ER+ CTCs using an exploratory cut-off of 0.3 CTC/ml. Fisher's exact test was used for the statistical analysis shown in the table. Waterfall plot displays the Epithelial+/ER+ CTCs counts. To avoid zero amputation on the logarithmic scale, 0.01 was added to each value before log-transformation. In the right panel, representative regions from 40x images showing Epithelial+/ER+ CTCs. Scale bar: 10 µm

2. Characterization of Epithelial+/HER2+ CTCs

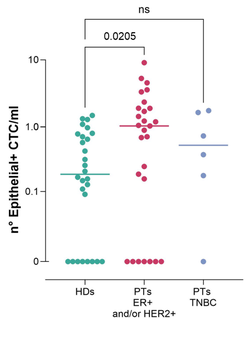
	n° (%) with CTC/ml < 0.3	n° (%) with CTC/ml ≥ 0.3	Total n°	P value
HDs	27 (96.4%)	1 (3.6%)	28	0.0084
HER2+ PTs	5 (55.6%)	4 (44.4%)	9	

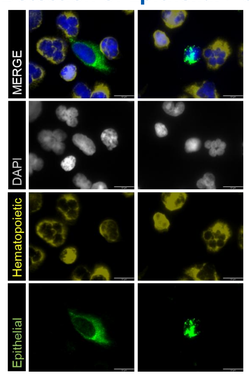




HDs and HER2+ PTs were discriminated for Epithelial+/HER2+ CTCs using an exploratory cut-off of 0.3 CTC/ml. Fisher's exact test was used for the statistical analysis shown in the table. Waterfall plot displays the Epithelial+/HER2+ CTCs counts. To avoid zero amputation on the logarithmic scale, 0.01 was added to each value before log-transformation. In the right panel, representative regions from 40x images showing Epithelial+/HER2+ CTCs. Scale bar: 10 µm

3. Detection of Epithelial+ CTCs





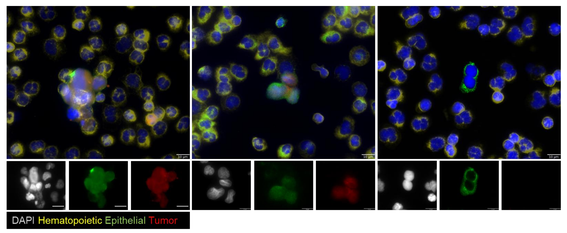
Epithelial+ CTCs, without any tumor marker expression, were detected and counted from HDs, ER+ and/or HER2+ PTs and TNBC PTs.

Dot plot shows the count for each sample; to avoid zero amputation, 0.01 was added to each value before log-transformation. Statistical analysis was performed with the non-parametric Kruskal-Wallis test.

Representative regions from 40x images displaying Epithelial+ CTCs detected in two different TNBC PTs are shown in the right panel. Scale bar: 10 µm

4. Identification of CTC clusters

	n° (%) with CTC cluster = 0	n° (%) with CTC cluster ≥ 1	Total n°	P value
HDs	28 (100.0%)	0 (0.0%)	28	0.0267
PTs	27 (81.8%)	6 (18.2%)	33	



CTC clusters were defined as aggregates of ≥ 2 neighbouring CTCs.

Their count was used to distinguish between HDs and MBC PTs. Fisher's exact test was used for the statistical analysis shown in the table.

40x fluorescence images show CTCs clusters in three PTs; higher magnification is shown in the images below. Enhanced contrast was applied for the images merge. Scale bar: 10 µm

Key findings and conclusions

- SmartBioSurface® slides allow the adhesion of both single and clustered CTCs without prior selection or enrichment.
- The proposed liquid biopsy workflow efficiently enables the single-cell characterization of ER and HER2 expression in CTCs from MBC patients.

Outlook

- Integrate multimodal assays (i.e. cytological and cytogenetical evaluation) on SmartBioSurface® slides.
- Potential use for monitoring metastatic cancer evolution and biomarker-related changes.

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References

[1] Alix-Panabières C. et al., *Cancer Discov*, 2021, Apr;11(4):858-873
[2] Visal T.H. et al., *Br J Cancer*, 2022, Jul;127(2):173-184
[3] Carbone R. et al., *Biomaterials*, 2006, 27(17):3221-9
[4] Krol I., Schwab F.D., Carbone R., Ritter M. et al., *Br J Cancer*, 2021, 125(1):23-27
[5] Mastromarino M.G., Parini S. et al., *Biomedicines*, 2023, 11(1):153
[6] Gole L. et al., Poster, 8th Digital Pathology & AI Congress Asia 2024

DISCLOSURE
G. Bianchini reports working with the following companies as an advisor, consultant, speaker, steering committee member or investigator: Amgen, Astra Zeneca, Chugai, Daiichi Sankyo, Eisai, Exact Science, Gilead, Helsinn, Lilly, Menarini Stemline, MSD, Neopharm Israel, Novartis, Pfizer, Roche, Sanofi, and Seagen.

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