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A NOVEL MULTIMODAL LIQUID BIOPSY WORKFLOW FOR THE CHARACTERIZATION OF CIRCULATING TUMOR CELLS IN METASTATIC BREAST CANCER

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TETHIS

INTRODUCTION

Circulating Tumor Cells (CTCs) enumeration can provide information on prognosis and treatment response in solid tumors [1].

Direct single-cell characterization of CTCs is challenging, despite technological advancements [2].

AIM

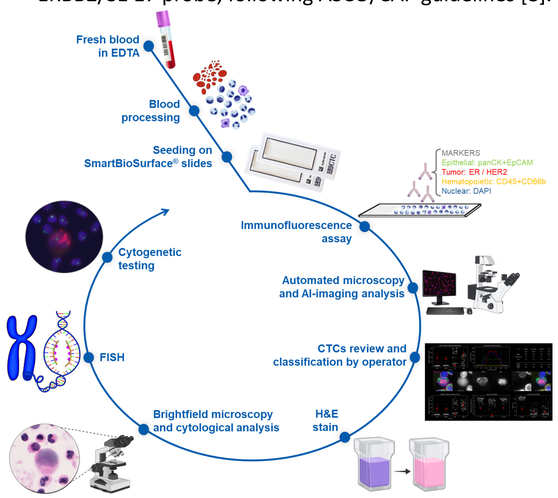
Characterize CTCs with an **innovative multimodal *in situ* approach** exploiting **SmartBioSurface® slides** [3-5], to overcome current limitations in the setting of **Metastatic Breast Cancer** (MBC).

METHODS

- **27 MBC patients** (PTs) (18 ER+, 7 HER2+, 2 ER+/HER2+) enrolled at San Raffaele Hospital (Milan, Italy) [6].
- **White blood cells** (WBCs) seeded on the **SmartBioSurface® slides**, at ~ 2.5 million WBCs/slide.
- **CTCs** analyzed by **immunofluorescence staining** [7] using antibodies against pan-cytokeratins, EpCAM, ER or HER2, and detected by **AI-driven imaging algorithm**.

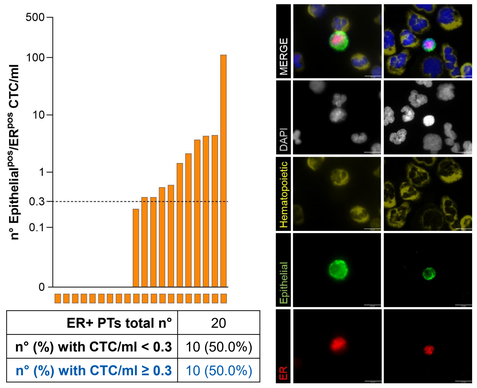
A representative **HER2+ MBC case** was further subjected to **sequential multimodal analysis**:

- **Hematoxylin and Eosin staining**, evaluated by a qualified cytologist;
- **Fluorescence In Situ Hybridization** (FISH) with ERBB2/SE 17 probe, following ASCO/CAP guidelines [8].



RESULTS

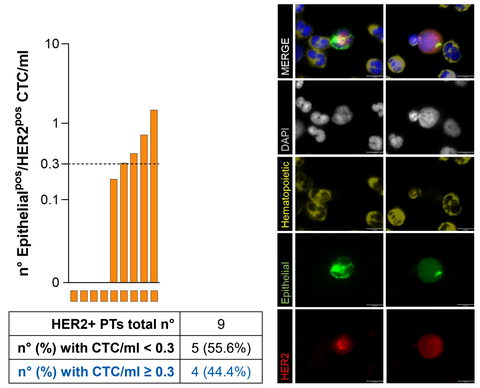
Epithelial^{pos}/ER^{pos} single CTCs



ER+ PTs total n°	20
n° (%) with CTC/ml < 0.3	10 (50.0%)
n° (%) with CTC/ml ≥ 0.3	10 (50.0%)

ER+ MBC samples were analyzed with immunofluorescence to detect CTCs co-expressing epithelial markers and ER.
Ordered bar plot shows Epithelial^{pos}/ER^{pos} CTCs counts on log-scale (0.01 was added to include zeros). The dotted line marks the exploratory threshold of 0.3 CTC/ml [7]; the table below summarizes PTs counts.
Right panel: 40× images of Epithelial^{pos}/ER^{pos} CTCs; scale bar: 10 µm.

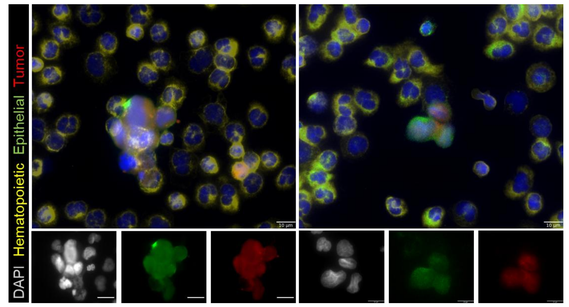
Epithelial^{pos}/HER2^{pos} single CTCs



HER2+ PTs total n°	9
n° (%) with CTC/ml < 0.3	5 (55.6%)
n° (%) with CTC/ml ≥ 0.3	4 (44.4%)

HER2+ MBC samples were analyzed with immunofluorescence to detect CTCs co-expressing epithelial markers and HER2.
Ordered bar plot shows Epithelial^{pos}/HER2^{pos} CTCs counts on log-scale (0.01 was added to include zeros). The dotted line marks the exploratory threshold of 0.3 CTC/ml [7]; the table below summarizes PTs counts.
Right panel: 40× images of Epithelial^{pos}/HER2^{pos} CTCs; scale bar: 10 µm.

CTC clusters



MBC PTs total n°	27
n° (%) with no CTC cluster	22 (81.5%)
n° (%) with CTC cluster	5 (18.5%)

CTC clusters (≥ 2 adjacent CTCs) were identified and counted.
40× images show clusters from two PTs; insert views below. Scale bar: 10 µm.
The table summarizes the detection rates of clusters. In 3 of 5 MBC PTs, CTC clusters were positive for the tumor marker expression, enabling their further characterization.

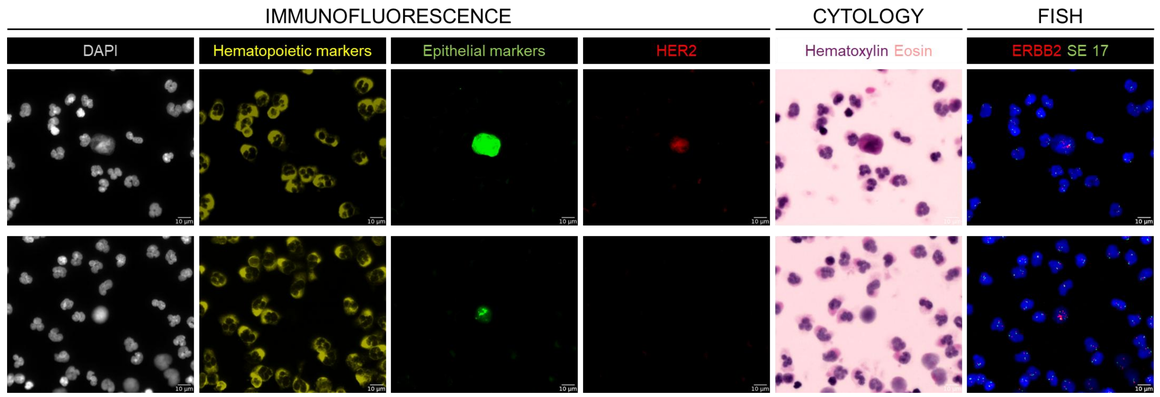
Multimodal analysis in a HER2+ MBC patient

A HER2+ MBC patient was chosen as a **case study** to demonstrate the feasibility of performing different and sequential analysis on the same SmartBioSurface® slide.

- Top line: Epithelial^{pos}/HER2^{pos} immunofluorescence-detected CTC.
- Bottom line: Epithelial^{pos}/HER2^{neg} immunofluorescence-detected CTC.

Both CTCs were subjected to subsequent Hematoxylin and Eosin staining, followed by FISH on the same slide.

- ➢ CTC on top line was evaluated as *suspected tumoral cell* by cytologist and displayed *ERBB2* amplification.
- ➢ CTC on bottom line showed *moderate atypical* features and displayed *ERBB2* amplification, even in the absence of HER2 protein expression by immunofluorescence.



CONCLUSIONS

The **liquid biopsy** workflow with **SmartBioSurface® slides** enables:

- ➢ **single-cell ER and HER2 profiling in individual and clustered CTCs**;
- ➢ **multimodal analysis (immunofluorescence, cytology, FISH)** without cell loss or degradation;
- ➢ the **capture of CTCs' heterogeneity** through integrated assays.

This method holds potential for **improving disease monitoring and personalized treatment strategies** in MBC.

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