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Technical Validation of a Novel Label-Independent Device to Capture CTCs

Gorges TM[1], Janning M[1], Kuske A[1], Wikman H[1], Müller V[2], Loges S[1], Riethdorf S[1], and Pantel K[1]

[1] Department of Tumour Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[2] Department of Gynecology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Universitätsklinikum Hamburg-Eppendorf

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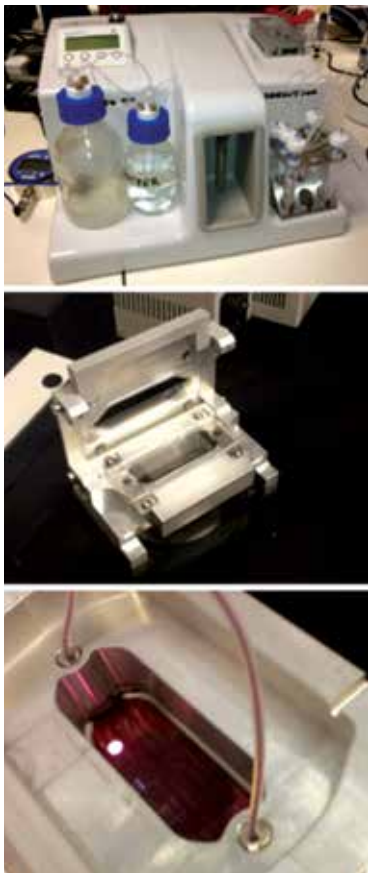
Background

Circulating tumor cells (CTCs) are tumor derived biomarkers found in the blood of cancer patients with a wide range of clinical utility. Precise enrichment of CTCs is challenging and frequently based on the epithelial cell adhesion molecule (EpCAM). However, a major consideration is the hypothesis that metastasis formation involves a shift of CTCs into a mesenchymal state (epithelial to mesenchymal transition, EMT) that would not allow the identification by epithelial markers. Hence, EpCAM-independent tumor cell enrichment strategies are needed for the comprehensive analysis of epithelial and EMT-associated CTC phenotypes.

Material and Methods

We used a novel marker-independent separation device (Parsortix) for tumor cell enrichment. Spiking experiments (MDA-MB-468, MDA-MB-231, MCF10A, and MCF-7) were done for staining validation and/or to test the recovery of the Parsortix system (EDTA or CellSave tubes). Immunocytochemistry (ICC) was performed for CTC enumeration. Besides, RNA analysis of pooled cells (MDA-MB-468) was done to test the viability of the cells (EDTA blood). Additionally, clinical blood samples from metastatic breast cancer patients (n = 35) were screened for CTCs and 19 were compared to the U.S. Food and Drug Administration-cleared CellSearch® system.

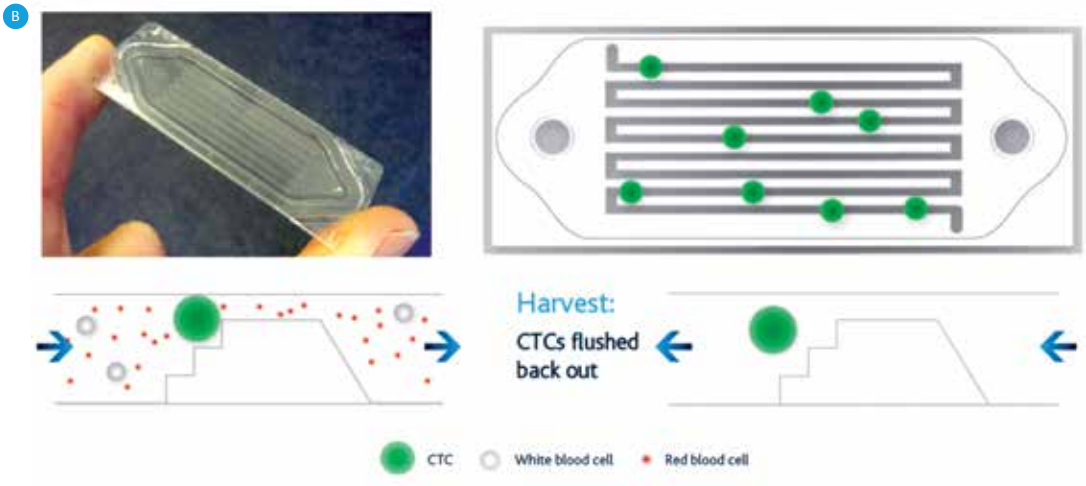
A

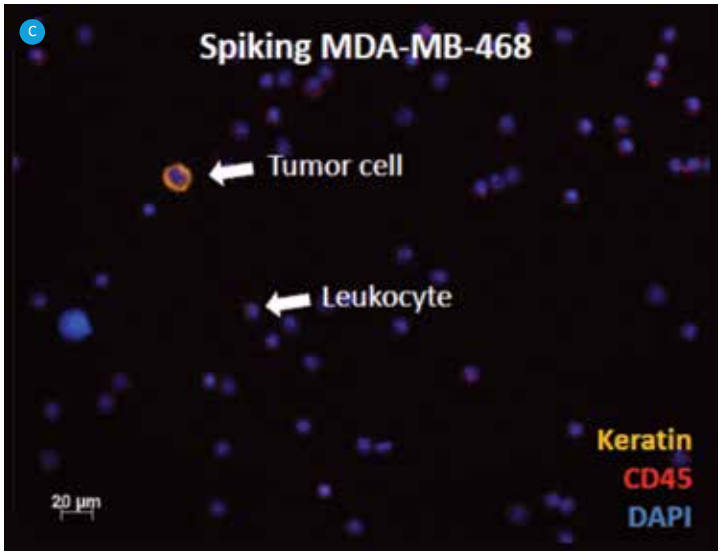


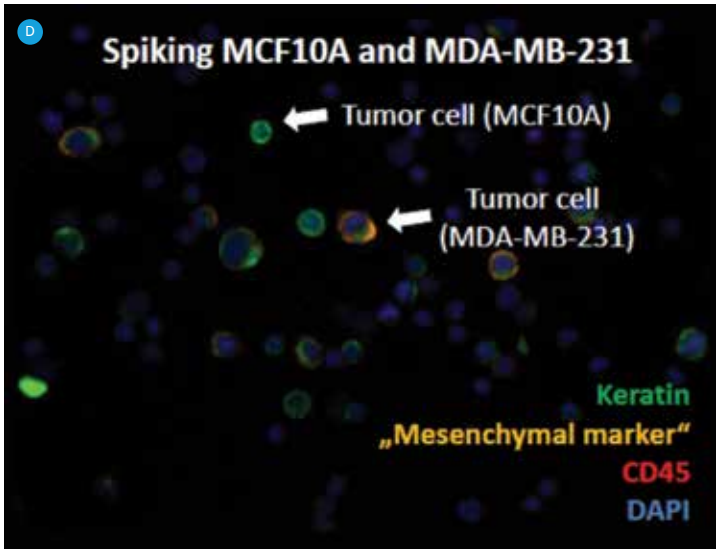
Parsortix System

A/B) The Parsortix separation device is a semi-automated system to capture cells based on their cell size (≥10 µm). The system allows the identification of cancer cells within the device but has also been developed to allow a release (harvest) of the captured CTCs outside the device. The harvesting process offers the potential for an easy and detailed molecular analysis of the CTCs.

B







EDTA Blood

Cells (cell line)	Recovery (after staining)
24 cells (MCF-7)	8 (33%)
10 cells (MCF-7)	3 (30%)
30 cells (MDA-MB-468)	12 (40%)
10 cells (MDA-MB-468)	4 (40%)

CellSave Blood

Cells (cell line)	Recovery (after staining)
30 cells (MDA-MB-468)	13 (43%)
30 cells (MDA-MB-468)	14 (47%)
30 cells (MDA-MB-231)	8 (27%)
30 cells (MDA-MB-231)	11 (37%)

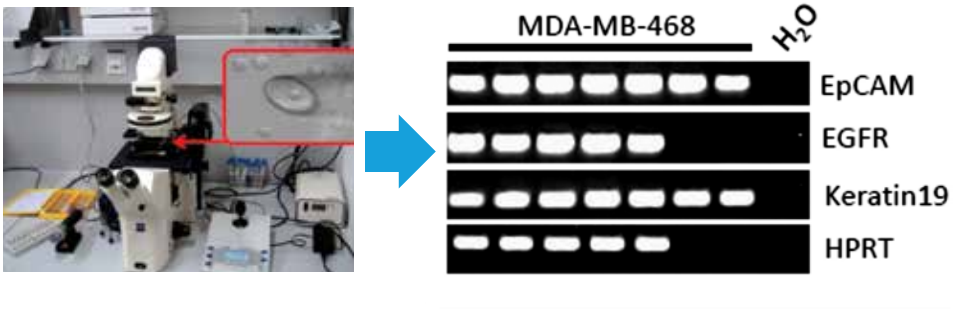
Spiking Experiments

C) Cells of the MCF-7, MCF10A, MDA-MB-468, MDA-MB-231, and SKBR-3 cell lines were spiked into blood from healthy donors (EDTA or CellSave blood), processed by Parsortix and harvested. Harvested cells were cytospun and stained for tumor specific (Keratin) as well as leukocyte specific (CD45) markers. DAPI was used for nuclear counterstain.

D) To prove that the Parsortix system is able to enrich mesenchymal-like CTCs a specific staining protocol for mesenchymal-like tumor cells (MDA-MB-231) was established.

RNA Analysis

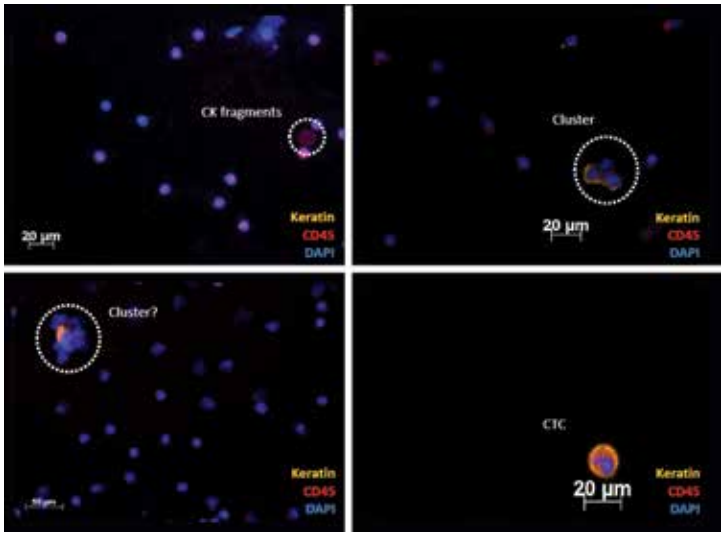
E



E) Breast cancer cells of the MDA-MB-468 cell line were spiked into blood from healthy donors and processed by Parsortix. Harvested cells were put on a glass slide and isolated by micromanipulation. Pooled cells (5 - 10) were transferred into PCR tubes followed by RNA isolation, cDNA synthesis, and PCR to prove that the cells are still functional for further molecular characterization.

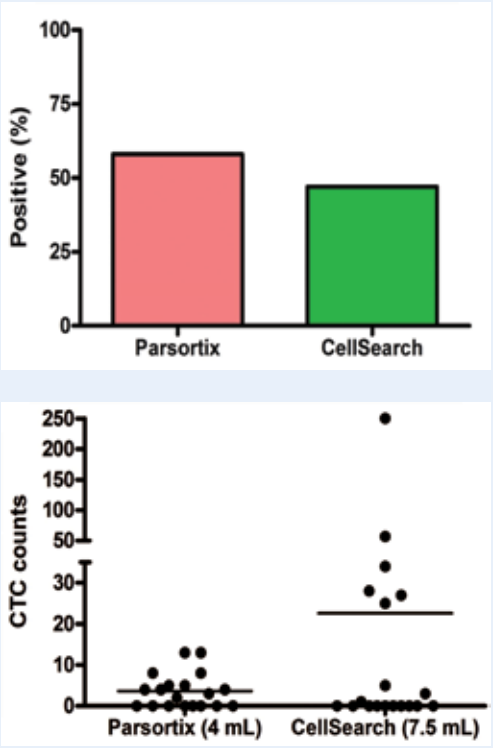
Cancer Patients

F



F) Analyzing clinical samples: CK fragments, single CTCs, and/or CTC cluster were found using Parsortix.

G



G) 35 breast cancer samples have been analyzed by Parsortix. 13/35 samples were positive for CTCs (37%, CTC range: 1-13 cells). 19 samples were processed in parallel to CellSearch®. 11/19 (58%) were positive by Parsortix (4 mL blood) (CTC range: 1-13 cells). 9/19 (47%) were positive by CellSearch® (7.5 mL blood) (CTC range: 1-250 cells). The concordance between both assays was 78%.

Conclusion

The Parsortix system is an effective device for the enrichment of epithelial- and/or mesenchymal-like CTCs. This system overcomes hurdles of label-dependent techniques since it is not based on antibody affinity capture. Using this system, tumor cells as well as tumor cell clusters are easily accessible and ready for further molecular analysis. Enumeration and molecular characterization of EpCAM-positive and EpCAM-negative CTC-subpopulations will help to understand the mechanisms of cancer biology and hasten the use of CTCs as “liquid biopsies”.

Acknowledgment

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www.angleplc.com

ANGLE NORTH AMERICA INC.
3711 Market Street
University City Science Center, 8th Floor
Philadelphia, PA 19104 USA

T: 215-966-6240
E: enquiries@angleplc.com

ANGLE EUROPE LTD.
3 Fredrick Sanger Road
Surrey Research Park
Guildford, Surrey GU2 7YD United Kingdom

T: +44 (0) 1483 685830
E: enquiries@angleplc.com

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