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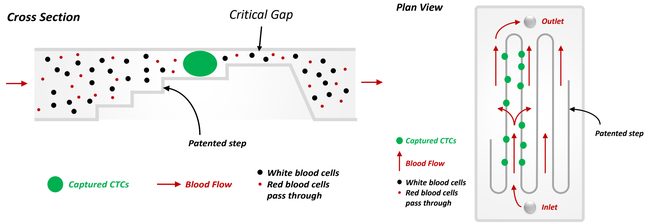
Identification of circulating tumor cells captured by the FDA cleared Parsortix® PC1 System from the peripheral blood of metastatic breast cancer patients using immunofluorescence and cytopathological evaluations

Mariacristina Ciccioli[1], Richard G. Moore[2], Kyu Kwang Kim[2], Negar Khazan[2], Joseph D. Khoury[3], M. Craig Miller[1], Anne-Sophie Pailhes-Jimenez[1]

[1]ANGLE Europe Limited, Guildford, UK; [2]Department of Obstetrics and Gynaecology, Wilmot Cancer Institute, University of Rochester Medical Center, Rochester, NY, USA; [3]Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.

Introduction

Circulating tumor cells (CTCs) captured from the blood of cancer patients may serve as a non-invasive source of tumor material to investigate tumor characteristics in real-time. The Parsortix® PC1 System, the first FDA-cleared medical device for the capture and harvest of CTCs from the peripheral blood of metastatic breast cancer (MBC) patients for use in subsequent user-validated downstream analyses, enables the epitope independent capture of CTCs with diverse phenotypes based on cell size and deformability.



The Parsortix® PC1 instrument is designed for use with a Parsortix® GEN3 Cell Separation Cassette (**Figure 1**). This study aimed to determine the proportion of MBC patients and self-declared female healthy volunteers (HVs) that had one or more CTCs identified in the population of cells harvested from their peripheral blood by the Parsortix® PC1 System using an immunofluorescence (IF) based assay for detection of epithelial CTCs followed by CTC identification using Wright-Giemsa staining and cytomorphological review.

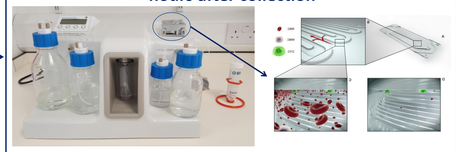
Figure 1. Parsortix® GEN3 Cell Separation Cassette layout and separation principle. The cassette contains a separation structure comprising a series of steps across which cells are forced to pass, leading them to a critical gap with a height of ~6.5 µm. The size and rigidity of CTCs prevent them from passing through the step structure and/or critical gap, and they are retained in the separation cassette together with a small number of residual white blood cells. The captured CTCs can then be harvested from the cassette and used for downstream analysis.

Materials and Methods

75 HVs and 77 MBCs patients included

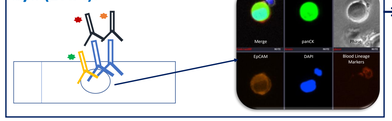
~8.6mL of blood collected from each subject into K_2EDTA tubes

Blood processed on Parsortix® PC1 Systems within 8 hours after collection

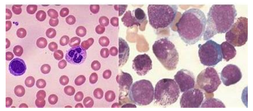


Harvested cells deposited onto a cytology slide using CytoSpin method

Staining, imaging and analysis using ANGLE's Epithelial IF assay: Pan-CK (FITC) + EpCAM (Cy3) + Blood lineage markers (Cy5) + Nuclear dye (DAPI).



Re-staining using Wright-Giemsa followed by brightfield imaging and cytology analysis



Peripheral blood from 75 HVs and 77 patients with MBC was prospectively collected into K_2EDTA tubes at the University of Rochester Medical Center for this study. Assay workflow is detailed in **Figure 2**. All laboratory testing and analysis was performed by operators blinded to the clinical status of each subject. First, CTCs were defined using IF staining as nucleated cells (DAPI+) that were positive for CK and/or EpCAM and negative for the blood lineage markers. Slides were then restained using Wright-Giemsa staining and morphological characteristics of malignant cells were used by a qualified pathologist to define and identify CTCs.

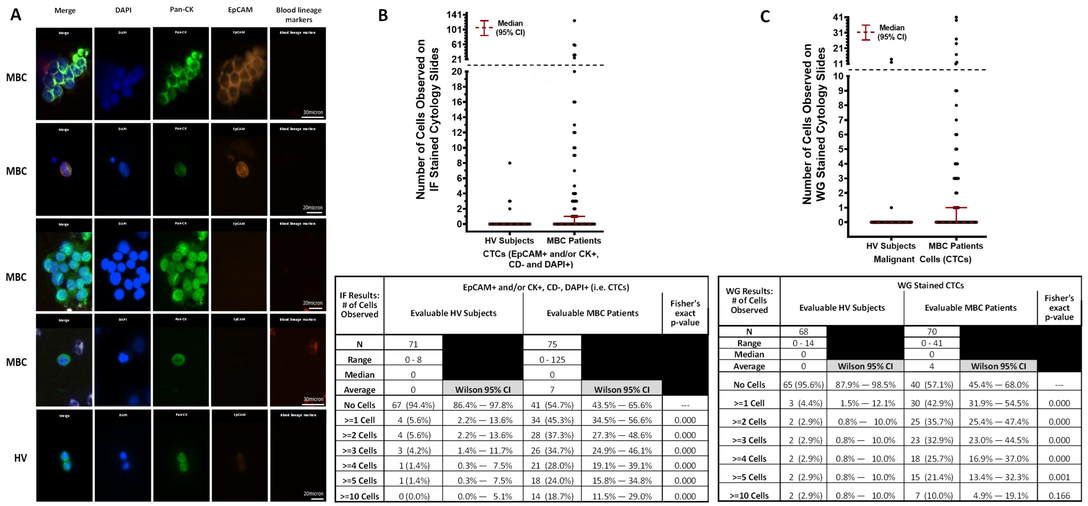
Figure 2. Schematic representation of the assay workflow. Blood collected from each subject (8.6±1.2mL) was processed on a Parsortix® PC1 System within 8 hours of collection. Cells harvested by the system were cytospun onto a charged slide and IF stained using an optimised antibody panel. The IF panel consisted of a nuclear dye (DAPI), positive selection markers targeting epithelial CTCs [Cytokeratins (CK) and EpCAM], and negative selection markers targeting white blood cells, such as lymphocytes, macrophages, granulocytes, monocytes, fibroblasts, and cells of megakaryoblastic potential. Stained slides were imaged using fluorescence microscopy and subsequently re-stained with Wright-Giemsa and analysed by a qualified pathologist using light microscopy.

Results

On the evaluable IF-stained slides (75 MBC and 71 HVs - **Figure 3A-B**):

- CTCs were identified in 45.3% of the MBC patients (range = 0 – 125, mean = 7) and in 5.6% of the HVs (range = 0 – 8, mean = 0).
- In the 34 MBC patients with one or more CTCs observed, 70.6% had only CK+, EpCAM- cells while the remaining 29.4% had at least 1 CK+, EpCAM+ cell. No EpCAM+, CK- CTCs were identified in either the MBC patients or HVs.

On the evaluable Wright-Giemsa stained slides (70 MBC and 68 HVs - **Figure 3C**), cells classified as CTCs by the pathologist were identified in 42.9% of the MBC patients (range = 0 – 41, mean = 4) and in 4.4% of the HVs (range = 0 – 14, mean = 0).



IF Results: # of Cells Observed	EpCAM+ and/or CK+, CD-, DAPI+ (i.e. CTCs) Evaluable HV Subjects		Evaluable MBC Patients		Fisher's exact p-value
N	71		75		
Range	0 - 8		0 - 125		
Median	0		0		
Average	0	Wilson 95% CI	7	Wilson 95% CI	
No Cells	67 (94.4%)	86.4% — 97.8%	41 (54.7%)	43.5% — 65.6%	---
>=1 Cell	4 (5.6%)	2.2% — 13.6%	34 (45.3%)	34.5% — 56.6%	0.000
>=2 Cells	4 (5.6%)	2.2% — 13.6%	28 (37.3%)	27.3% — 48.6%	0.000
>=3 Cells	3 (4.2%)	1.4% — 11.7%	26 (34.7%)	24.9% — 46.1%	0.000
>=4 Cells	1 (1.4%)	0.3% — 7.5%	21 (28.0%)	19.1% — 39.1%	0.000
>=5 Cells	1 (1.4%)	0.3% — 7.5%	18 (24.0%)	15.8% — 34.8%	0.000
>=10 Cells	0 (0.0%)	0.0% — 5.1%	14 (18.7%)	11.5% — 29.0%	0.000

WG Results: # of Cells Observed	WG Stained CTCs Evaluable HV Subjects		Evaluable MBC Patients		Fisher's exact p-value
N	68		70		
Range	0 - 14		0 - 41		
Median	0		0		
Average	0	Wilson 95% CI	4	Wilson 95% CI	
No Cells	65 (95.6%)	87.9% — 98.5%	40 (57.1%)	45.4% — 68.0%	---
>=1 Cell	3 (4.4%)	1.5% — 12.1%	30 (42.9%)	31.9% — 54.5%	0.000
>=2 Cells	2 (2.9%)	0.8% — 10.0%	25 (35.7%)	25.4% — 47.4%	0.000
>=3 Cells	2 (2.9%)	0.8% — 10.0%	23 (32.9%)	23.0% — 44.5%	0.000
>=4 Cells	2 (2.9%)	0.8% — 10.0%	18 (25.7%)	16.9% — 37.0%	0.000
>=5 Cells	2 (2.9%)	0.8% — 10.0%	15 (21.4%)	13.4% — 32.3%	0.001
>=10 Cells	2 (2.9%)	0.8% — 10.0%	7 (10.0%)	4.9% — 19.1%	0.166

Figure 3. CTC identification by IF and Wright-Giemsa staining. (**A**) Representative images of CK+, EpCAM +/- CTCs and CTCs clusters identified in MBC patients and HV subjects (Cytokeratins (FITC) in green, EpCAM (Cy3) in orange, Blood lineage markers (Cy5) in red, Nucleus (DAPI) in blue). Dot plots show median ± 95% Confidence Interval (CI) of the number of CTCs identified in each MBC and HV donor by (**B**) IF and (**C**) Wright-Giemsa staining. A statistically higher number of CTCs was found in MBC patients compared to HVs using both downstream assays ($p \geq 0.001$, Median test). Tables show number of donors included in each cohort (N), range, median and average number of CTCs identified within each cohort, percentage of donors with ≥1, ≥2, ≥3, ≥4, ≥5, ≥10 CTCs identified, Wilson 95% CI and *p* values obtained using Fisher's exact test for statistically significant differences between MBC and HVs in (**B**) IF and (**C**) Wright-Giemsa stained samples.

Conclusions

- **ANGLE's Parsortix® PC1 System can capture and harvest CTCs from a significantly larger proportion of MBC patients compared to HVs and the harvested cells can be successfully evaluated using both IF staining and Wright-Giemsa cytomorphological assessment.**
- **High proportion of CTCs did not express EpCAM, further highlighting limitations of using EpCAM-based approaches to capture CTCs.**
- **It was demonstrated at ANGLE that the use of cytospin causes up to 70% cell loss. Therefore, these results substantially understate the number of CTCs harvested by Parsortix® PC1 instrument. ANGLE is currently developing alternative harvest processes with limited cell loss for downstream imaging assays.**