Epithelial and Mesenchymal CTC Detection in Triple Negative Breast Cancer Patients using ANGLE's Parsortix® System

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Introduction

Limited Circulating Tumor Cells (CTCs) research is available in Triple Negative Breast Cancer (TNBC) due to the inability of epitope-dependent CTC enrichment platforms to capture CTCs in a sufficient proportion of patients, especially when evaluating non-metastatic cancer patients (Stage I-III).

ANGLE developed a Research Use Only (RUO) workflow comprising the use of the Parsortix® system, an epitope-independent microfluidic device that isolates and harvests CTCs from blood based on their size and lack of deformability, followed by staining using ANGLE's Immunofluorescence (IF) assay for identification of epithelial and mesenchymal CTCs. In this study, performance of the assay was assessed in TNBC.

For Research Use Only. Not for use in diagnostic procedures.

Workflow

Peripheral blood samples were collected from 12 TNBC patients into Streck Cell-Free DNA Blood Collection Tubes (BCTs) and used in this study. The blood samples were processed between 96 and 144 hours from the time of the collection of the blood using Parsortix® systems for the capture and harvest of CTCs. The captured cells were stained using a multiplex IF assay for the identification and characterization of both epithelial and mesenchymal CTCs.

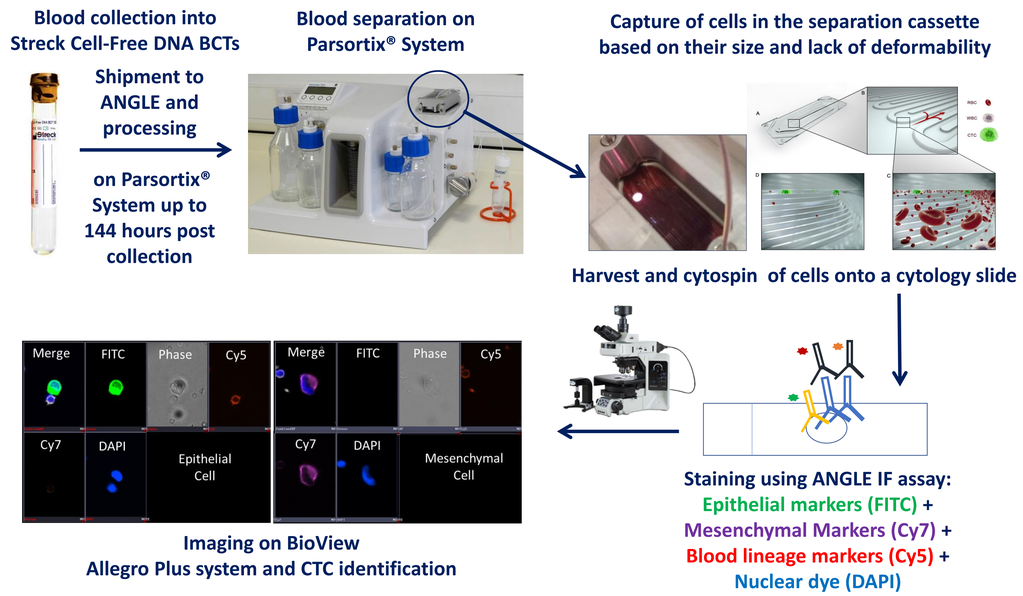

Figure 1. Schematic representation of the assay workflow. Peripheral blood (7.5 mL) was drawn into Streck Cell-Free DNA BCTs from 12 TNBC patients and processed on Parsortix® systems between 96 and 144 hours post collection. Cells captured in the Parsortix® cell separation cassettes due to their larger size and lower compressibility compared to other blood components were harvested, cytospun onto slides, and immunofluorescently stained for detection of epithelial and mesenchymal CTCs. Slides were imaged using a BioView Allegro Plus imaging system. CTCs were defined as epithelial (FITC+, Cy7-, Cy5-, DAPI+), mesenchymal (FITC-, Cy7+, Cy5-, DAPI+), or Epithelial-to-Mesenchymal transitioning (FITC+, Cy7+, Cy5-, DAPI+).

Results

- 83% (10/12) of the TNBC patients included in this study had ≥1 CTC identified. Interestingly, CTC positivity rate in Stage IIIa and Stage IV patients was similar (88% vs 75%, respectively).
- CTC clusters (consisting of 2 to 68 cells per cluster) were observed in 90% (9/10) of the CTC positive patients.
- Phenotypically, a large proportion of the CTCs identified expressed only mesenchymal markers (80%), with the remainder expressing both epithelial and mesenchymal markers (20%).

A		TNBC (all)	Stage IIIa	Stage IV
N		12	8	4
Donors ≥ 1 CTC (N and %)		10/12 (83%)	7/8 (88%)	3/4 (75%)
CTC Range		1–282	1–282	32–72
CTC mean		44	46	39
CTC median		17	13	41
Donors Phenotype	Epithelial	0/10 (0%)	0/7 (0%)	0/3 (0%)
	Mesenchymal	8/10 (80%)	6/7 (86%)	2/3 (67%)
	Transitioning (EMT)	2/10 (20%)	1/7 (14%)	1/3 (33%)
Donors ≥ 1 CTC Cluster (N and % over donors ≥ 1 CTC)		9/10 (90%)	6/7 (86%)	3/3 (100%)
N clusters per donor (range)		1–50	1–50	1–14
N CTC per cluster (range)		2–68	2–68	2–30

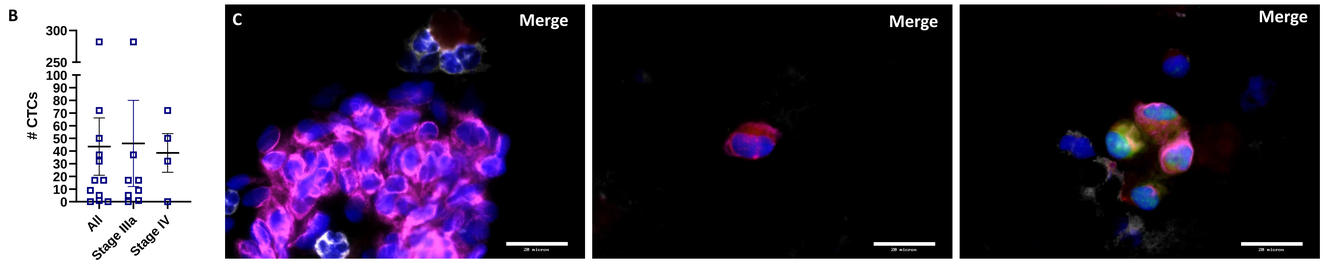

Figure 2. CTC identification and phenotyping. (A) Table showing number of donors included in each cohort (N), N and percentage (%) of donors with ≥1 CTC, range, mean and median of CTCs captured across donors, donors' CTC phenotype, number and percentage of donors with ≥1 CTC cluster, range of CTC clusters per donor, and range of number of CTCs per cluster; **(B)** Dot plot showing the number of CTCs (with mean ± SEM) found in each patient group; **(C)** Representative images of a cluster of mesenchymal CTCs (Left), a single mesenchymal CTC (Middle) and a cluster of transitioning (EMT) CTCs (Right). Merge colors: Epithelial markers (FITC) in green, Mesenchymal markers (Cy7) in magenta, Blood lineage markers (Cy5) in white, Nuclear dye (DAPI) in blue.

ANGLE's IF assay can be multiplexed into a five-channel assay, enabling not only the identification of both epithelial and mesenchymal CTCs, but also the opportunity to investigate other targets of interest on CTCs. Some examples of the further characterization of the CTCs using the 5th channel are shown below.

Epithelial markers (FITC) + Mesenchymal Markers (Cy7) + Blood lineage markers (Cy5) + Nuclear dye (DAPI) + Target of interest (Cy3)

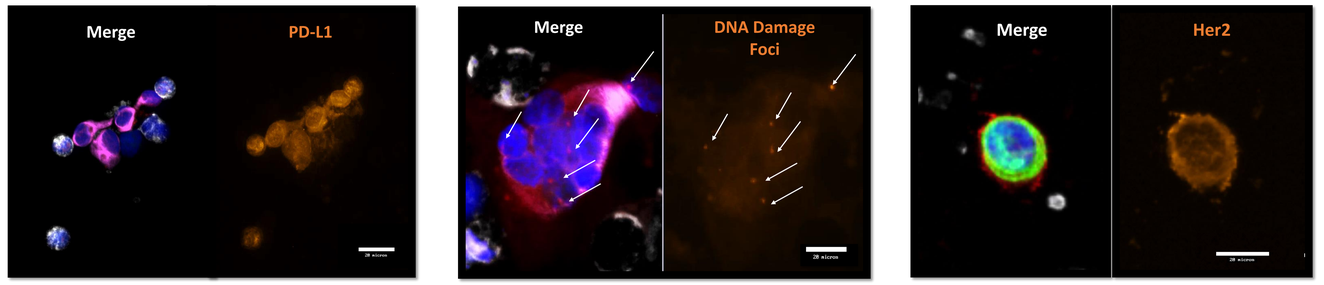

Figure 3. Representative images of CTCs or cancer cells lines identified using ANGLE's Parsortix® system and IF assay combined with alternative markers in the Cy3 channel: PD-L1 expression on CTCs (Left), DNA Damage Marker as foci in CTCs (Middle), Her2 expression on SKBR3 cell (Right). Merge colors: Epithelial markers (FITC) in green, Mesenchymal markers (Cy7) in magenta, Blood lineage markers (Cy5) in white, Nuclear dye (DAPI) in blue, Optional marker (Cy3) in orange.

Conclusions

- ANGLE's workflow can detect a considerable number of CTCs in both Stage IIIa and Stage IV TNBC patients, with a higher observed positivity rate compared to previous studies using epitope-dependent systems (~82% vs ~25%).
- ANGLE's workflow has the added advantage of being able to process blood between 96 and 144 hours post collection, allowing for the shipment of samples for centralised analysis in support of global clinical trials.
- ANGLE's multiplex IF assay offers the ability to phenotype CTCs using both epithelial and mesenchymal marker expression while enabling the evaluation of other markers of interest.