Combined epitope-independent microfluidic isolation and advanced staining of circulating tumour cells isolated from metastatic prostate cancer blood samples.

David Greaves [1], Aaron Cottingham [1], Laura Kaja [1], Chloe Goodwin [1], Morgan Spode [1], Aarabhi Varatharajah [1], Mariacristina Ciccioli [1], Anne-Sophie Pailhes-Jimenez [1]

[1] ANGLE Europe Limited, 10 Nugent Road, Surrey Research Park, Guildford, Surrey GU2 7AF United Kingdom

EACR Conference LIQUID BIOPSIES

12 - 14 November 2024, Lyon

#31

Introduction

The isolation of Circulating tumour cells (CTCs) from blood samples holds immense potential for cancer screening, management and monitoring. However, epitope-dependent capture systems limit detection of mesenchymal CTCs, and CTCs undergoing epithelial-to-mesenchymal transition (EMT) **(Figure 1).** Combining the ANGLE Parsortix® system and Portrait®+ CTC staining kit addresses this need: utilising epithelial and mesenchymal markers for identification, enumeration, and phenotyping of CTCs isolated through the Parsortix® system, a label-independent microfluidic device which isolates CTCs based on size and compressibility.

For Research Use Only. Not For Use In Diagnostic Procedures.

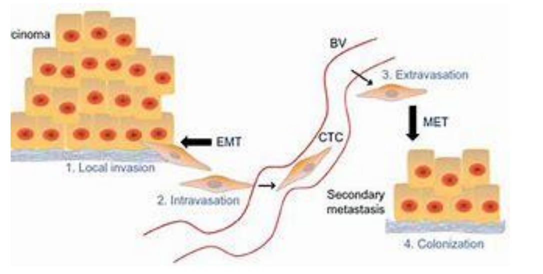


Figure 1. Epithelial to mesenchymal transition. Figure taken from Guttilla Reed I., *Cell Health and Cytoskeleton.* 2015

Workflow

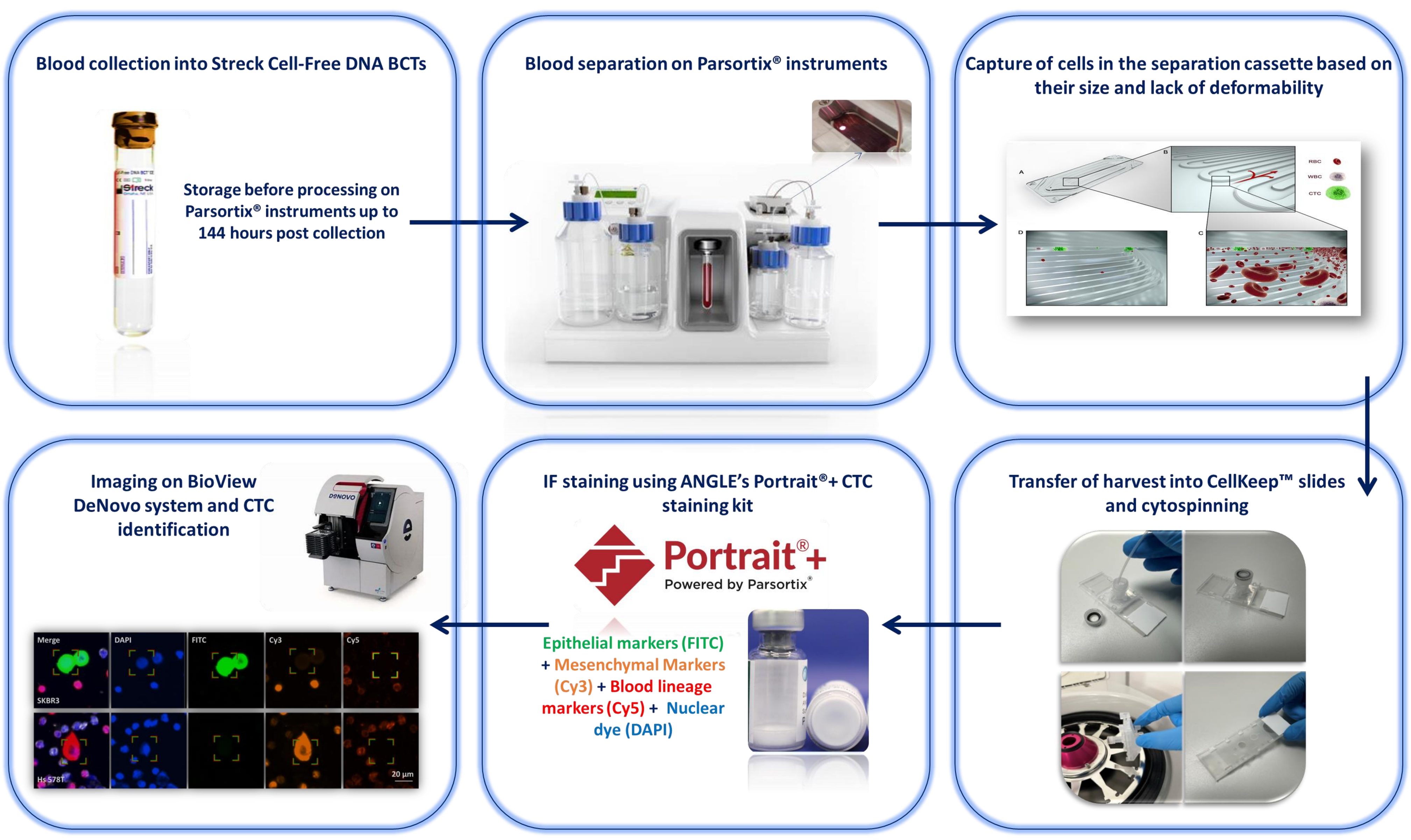


Figure 2. Schematic representation of the assay workflow.

Analytical performance was established using Healthy Volunteer's (HV) blood samples spiked with either epithelial, mesenchymal or epithelial-to-mesenchymal transitioning (EMT) cancer cell lines, while clinical performance was established using blood samples from Metastatic Prostate Cancer (PCa) patients. In both cases (**Figure 2**):

- Peripheral blood was drawn into Streck Cell-Free DNA tubes and stored for up to 144 hours from collection before processing.
- Blood samples were processed on Parsortix® instruments, a microfluidic device capable of capturing and harvesting CTCs from bodily fluids based on cell size and lack of deformability, employing a separation cassette (GEN3P6.5) comprising a series of steps leading to a smaller critical gap. Most of the common blood cells and components pass across the critical gap, while CTCs are retained in the separation cassette due to their size and rigidity.
- Captured CTCs were removed from the separation cassette and cytocentrifuged on ANGLE's CellKeep™ slides to maximize cell retention.
- Slides were stained using ANGLE's Portrait®+ CTC staining kit, a freeze-dried antibody mixture, comprising a nuclear dye (Hoechst) and antibodies against epithelial markers (FITC), mesenchymal markers (Cy3), and blood lineage markers (Cy5), including antigens expressed by blood cells such as lymphocytes, macrophages, granulocytes, monocytes, fibroblasts, and cells of megakaryoblastic potential.
- Stained slides were imaged using a BioView DeNovo imaging system.

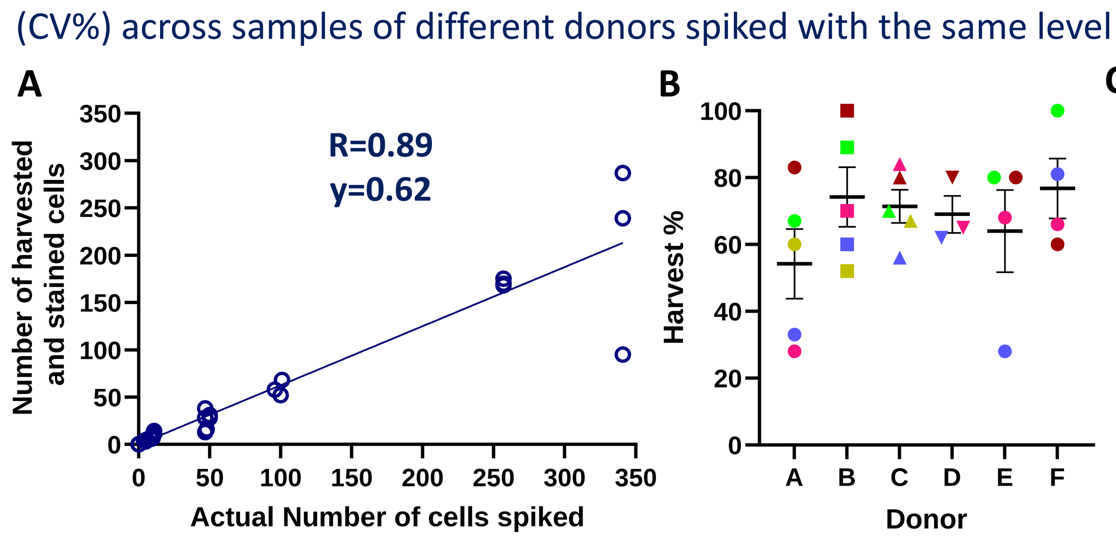
Analytical Results

Linearity, Accuracy and Precision were established by assessing the quantitative recovery of between 0 and ~250 H226 cells spiked into 7.5 mL of HVs' blood drawn in Streck Cell-Free DNA BCTs:

Linearity: a linear relationship between the number of H226 cells spiked and the number of harvested cells was confirmed, with an R^2 of 0.89 and deviation from linearity within ±28.6% (**Figure 3A**).

Accuracy: how close or dispersed the number of harvested and stained cells is to the number of spiked cells in blood. Mean accuracy across six donors for the end-to-end workflow was 68% (**Figure 3B**).

Precision: the reproducibility of the experiment and indicates how close or dispersed the percentage of harvested and stained cells is between samples as a measure of variability. Coefficient of variation (CV%) across samples of different donors spiked with the same level of cells was <37% (**Figure 3C**).



C

Donor	A	B	C	D	E	F
N	5	5	5	3	4	4
Mean	54.2	74.2	71.4	69.0	64.0	76.8
CV (%)	42.9	26.9	15.5	14.0	38.5	23.2

Figure 3. Quantitative analytical performance evaluation. (A) Graph shows actual number of H226 cells spiked (x-axis) vs. the actual number of harvested and stained cells (y-axis) across all spike levels for six donors. **(B)** Dot plot shows Mean ± Standard Error of the Mean (SEM) for the percentage of harvested and stained H226 cells per donor at each spike level. **(C)** Table shows number of samples (N), mean % harvested cells and CV% by HV donor.

Results from Patient Samples

The workflow was performed on blood samples collected from 47 PCa patients and 12 HV donors to assess the number of epithelial, mesenchymal and EMT CTCs, and CTCs clusters captured (**Figure 4**):

- 64% (30/47) of the PCa patients included in this study had ≥1 CTC identified and CTC clusters (consisting of 2 to 200 CTCs per cluster) were observed in 67% (20/30) of the CTC positive patients.
- Phenotypically, 77% of the patients had only mesenchymal CTCs identified, and 20% had a combination of mesenchymal, and epithelial or EMT CTCs
- One patient (3.3%) had exclusively EMT CTCs, and none had exclusively epithelial CTCs.
- Only one cluster of 7 mesenchymal CTCs was identified in 1/12 (8%) HVs.

A	PCa
N	47
Donors with ≥1 CTC	30/47 (64%)
Donors with ≥5 CTC	22/47 (47%)
Median	7
Mean	62
Range	1-262
Donors with ≥1 CTC Cluster	20/30 (67%)
Cluster size (range CTC)	2-200
Cluster per donor (range)	1-47
Donors with only EMT CTCs	3.3% (1/30)
Donors with only Mesenchymal CTCs	76.7% (23/30)
Donors with Mz and EMT CTCs	6.7% (2/30)
Donors with Mz and Ep CTCs	13.3% (4/30)

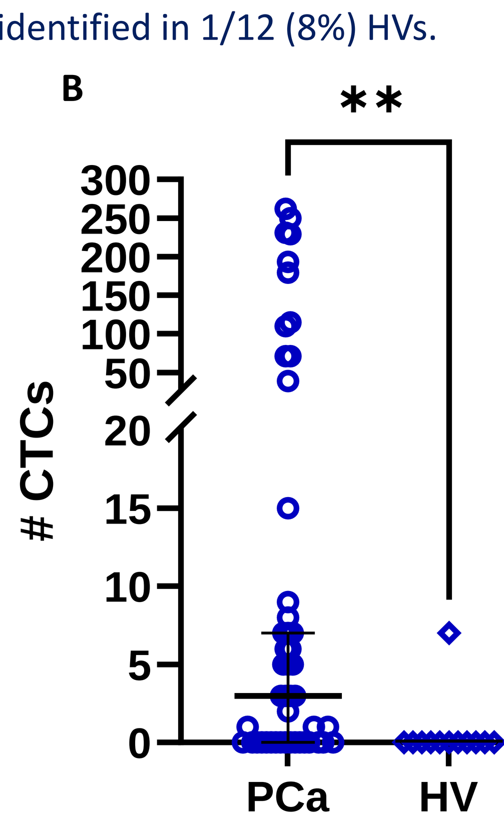


Figure 4. CTC identification and phenotyping in PCa patients and HV donors. (A) Table showing number of donors included in the cohort (N), N and percentage (%) of donors with ≥1 CTC, range, mean and median of CTCs (excluding negative donors), number and percentage of donors with ≥1 CTC cluster, range of CTC clusters per donor, range of number of CTCs per cluster and donors' CTC phenotype; **(B)** Dot plot showing the number of CTCs (median ± 95% CI) found for all patients and HV donors. (** p≤0.005, Mann Whitney Test)

Patient Longitudinal Analysis

The patients from the initial cohort were monitored, with each providing up to five additional blood draws (maximum of six draws per patient). These draws were taken at an interval of one to five months dependent on the individual patient's treatment schedule.

All blood draws were processed and analysed using the above workflow, with CTC data compared to patient treatment data and reported clinical status. Only donors who provided at least four consecutive draws (N=20) are included in this analysis cohort (study ongoing).

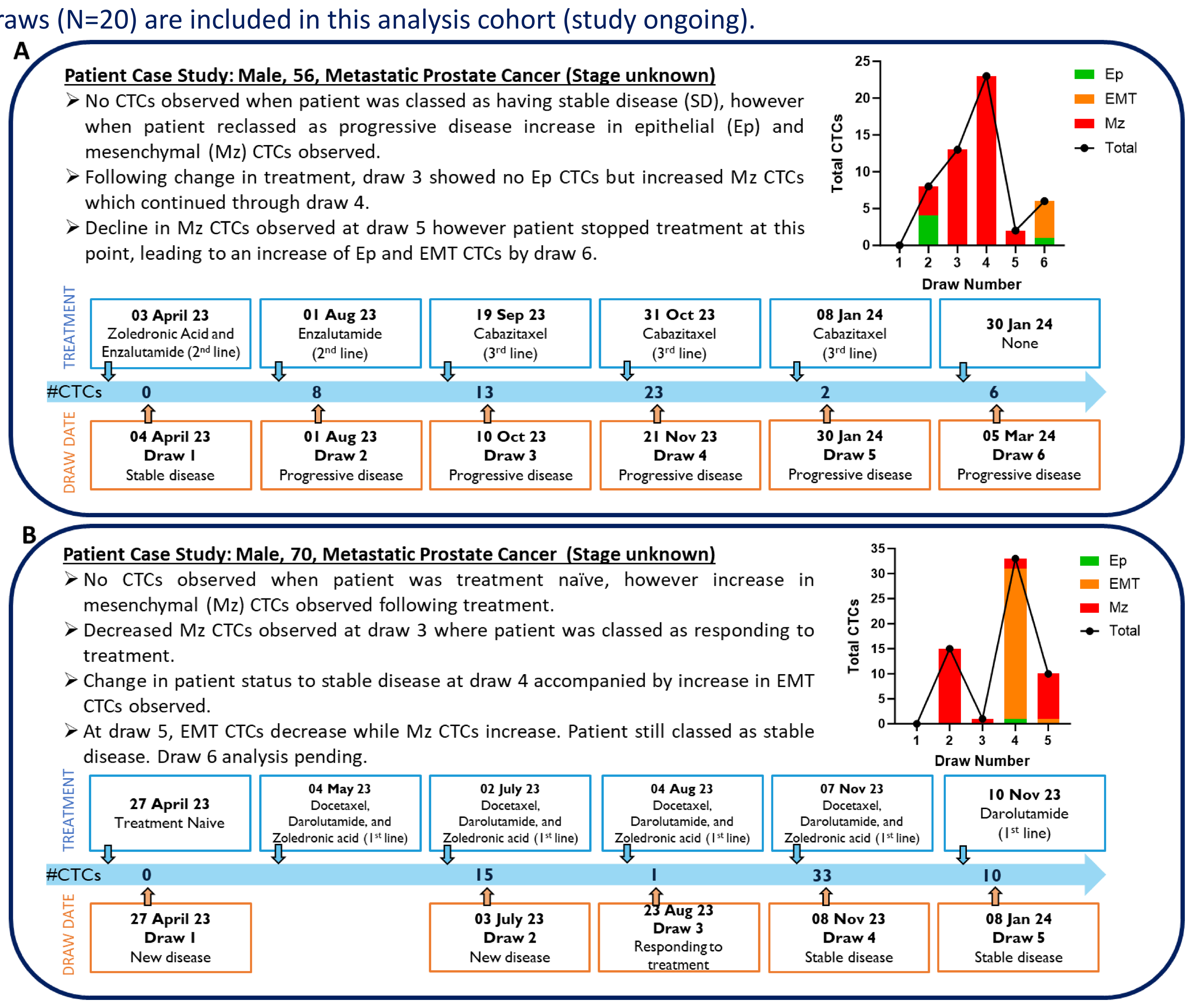


Figure 5. CTC identification and phenotyping in PCa patients. (A+B) Case studies of metastatic PCa patients, with changes in CTC numbers and phenotype identified across draws and treatment.

Patient CTCs: Representative Images

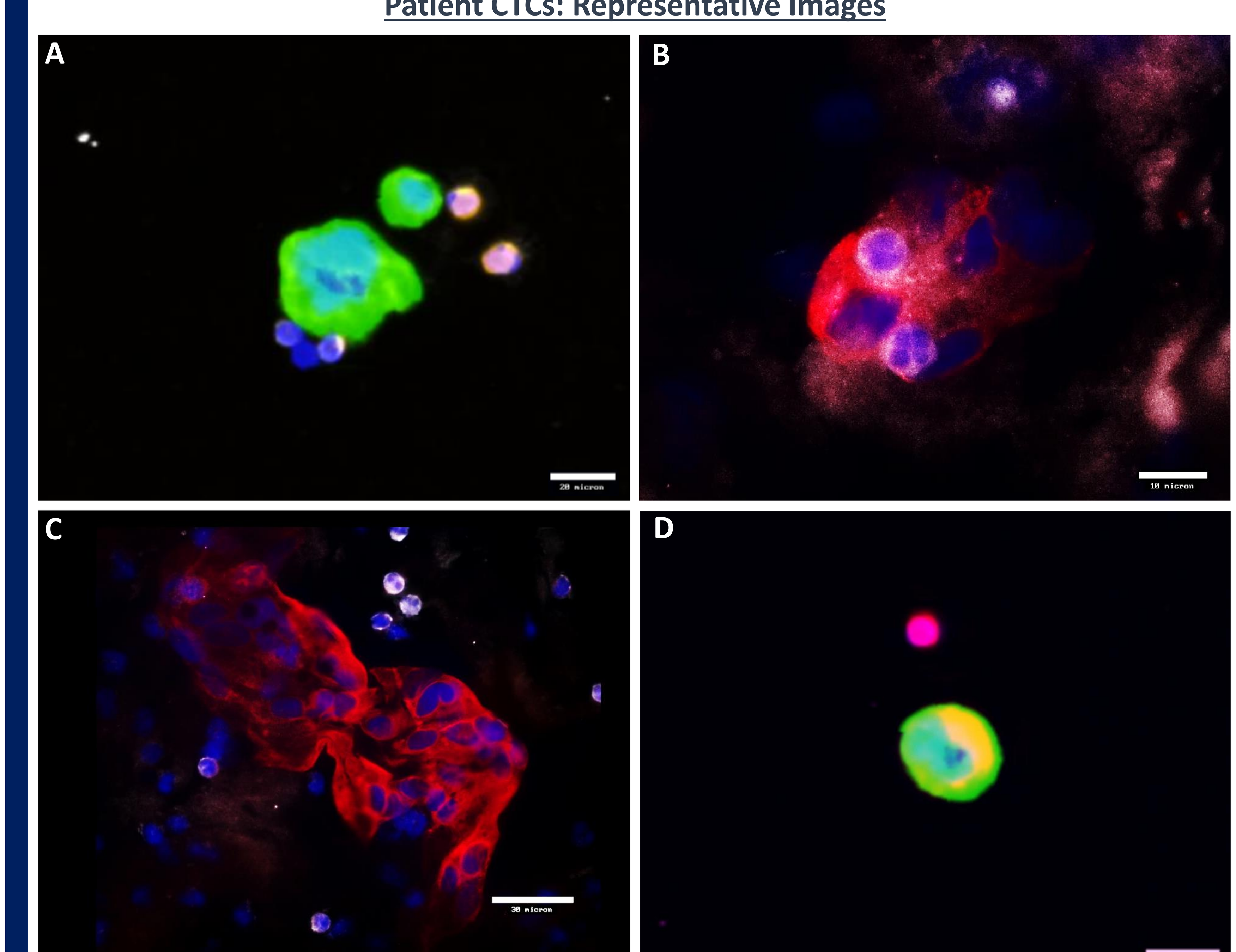


Figure 6. Representative images of IF stained CTCs found in PCa patient slides. (A) Representative image of epithelial CTCs. **(B)** A small mesenchymal CTC cluster with PBMC infiltrate. **(C)** A large cluster of mesenchymal CTCs. **(D)** A single epithelial to mesenchymal transitioning CTC.
Merge colors: Epithelial markers (FITC) in green, Mesenchymal markers (Cy3) in Red, Blood lineage markers (Cy5) in white, Nuclear dye (DAPI) in blue.

Conclusions

This study demonstrates the utility of combining Parsortix separation with Portrait+ IF staining for both cancer screening and longitudinal patient monitoring. The study also highlights the importance of epitope-free CTC isolation systems for the processing of blood from PCa patients. An epithelial only approach would fail to capture the full range of CTCs present.

ANGLE's Portrait®+ CTC staining kit to detect and phenotype CTCs, combined with ANGLE's Parsortix® system, provides an efficient, standardized solution for the characterization of multiple CTC phenotypes.