# Morphologic Variation of Circulating Tumor Cells in Metastatic Breast Cancer Patients Visualized by Immunofluorescent Stain Patterns

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#### **ABSTRACT**

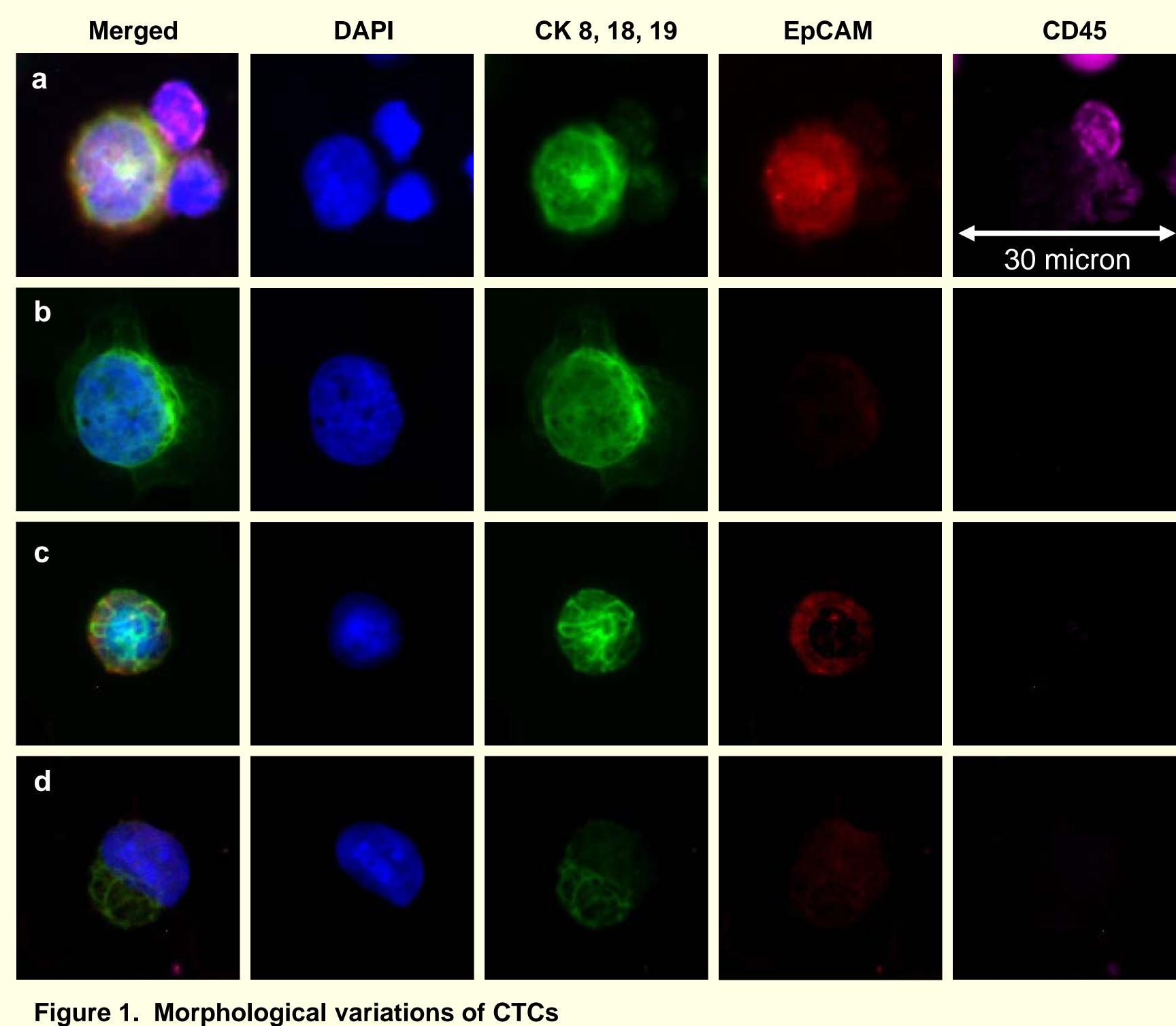
Microfiltration is an increasingly popular method for isolating circulating tumor cells (CTCs) from the peripheral blood of patients with solid tumors. This "liquid biopsy" is a rapid and non-invasive method to obtain tumor cells for disease subtyping and staging. Here we present a use of CellSieve™ microfilters to characterize CTCs based on immunofluorescent staining patterns of their cytokeratin filamentation and EpCAM expression.

Our results illustrate that disseminated CTCs have high phenotypic heterogeneity. Detailed sub-grouping analysis and patient tracking of these phenotypes shows correlation between stage of disease, progression of disease and chemotherapeutic response.

#### INTRODUCTION

CTCs are cells that originate from a primary solid tumor and are found transiting the circulatory system. CTCs can be used to monitor therapy response and predict outcome.<sup>1-4</sup> Size exclusion is a technique for isolating CTCs irrespective of their surface marker expression.<sup>2-4</sup>

CellSieve™ microfilters are lithographically fabricated membranes with high porosity, precise pore dimensions, and precise pore distribution. We previously reported that CellSieve™ rapidly and efficiently isolates CTCs from whole peripheral blood, using fluorescent antibody stain as the detection platform. Here we further characterized CTCs based on their morphological characteristics, consistent with standard histological stain techniques, and compared with patient disease and treatment status. CTC subtyping by phenotypic determinates may aid identifying the CTCs cellular status for diagnosis, prognosis and therapy determination.<sup>2-4</sup>



- (a) CTC with high EpCAM expression bound to two white blood cells
- (b) CTC with high nucleus-to-cytoplasm ratio and no EpCAM
- (c) Small CTC with EpCAM
- (d) CTC with low cytokeratin and low EpCAM

# **MATERIAL & METHODS**

Blood samples from metastatic breast cancer patients were provided by University of Maryland and Fox Chase Cancer Center. Microfilters were fabricated with 8 micron diameter pores in a uniform array over a 9 mm diameter area. 7.5 mL of whole blood was diluted 1:1 in a fixative and filtered through CellSieve<sup>TM</sup> microfilters (~3 min). CTCs collected were then fixed, permeabilized, and stained with DAPI, and antibodies specific to CK 8, 18 and 19 (FITC), EpCAM (PE), and CD45 (DyLight 649). Cells without CD45 staining were classified by their morphology, nuclear integrity and the presence of cytokeratin and EpCAM.

### RESULTS

- Assay time was <2 hours.
- CTCs were easily identified by traditional fluorescent stains (Figs. 1a-d).
- CTCs were identified with either high, low, or no EpCAM expression (Figs. 1-3).
- CTCs were found to have variable Cytokeratin expression and patterns (Figs. 1-3).
- Apoptotic CTCs could be identified by their spotted morphology (Figs.2a-c).
- Cell clusters, cells in division, and cells with varying EpCAM expression could be identified and classified for comparative analysis (Figs. 3a-c).
- CTC morphology varied by disease stage and therapy status (Figs. 2a-c).

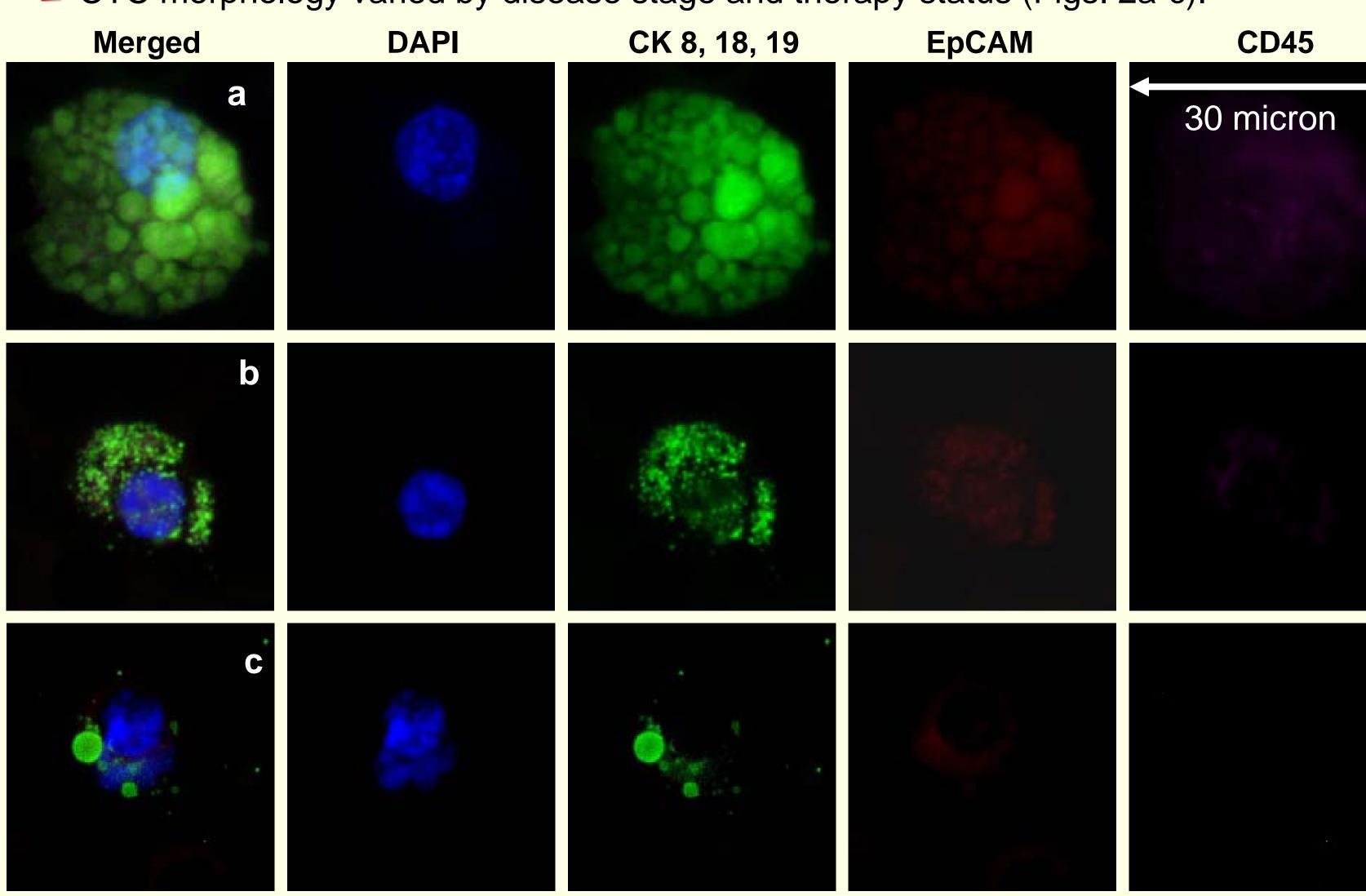


Figure 2. Apoptotic CTCs. (a) Early stage. (b) Late stage. (c) Very late stage.

## CONCLUSIONS

- Microfiltration captures CTCs regardless of EpCAM expression.
- Microfiltration captures weakened and apoptotic CTCs.
- CTCs captured by CellSieve™ are easily characterized by phenotypic morphology.
- CTC phenotypes differ according to treatment and disease stage.
- Further longitudinal study of patient CTCs is expected to provide additional information about patient assessment.

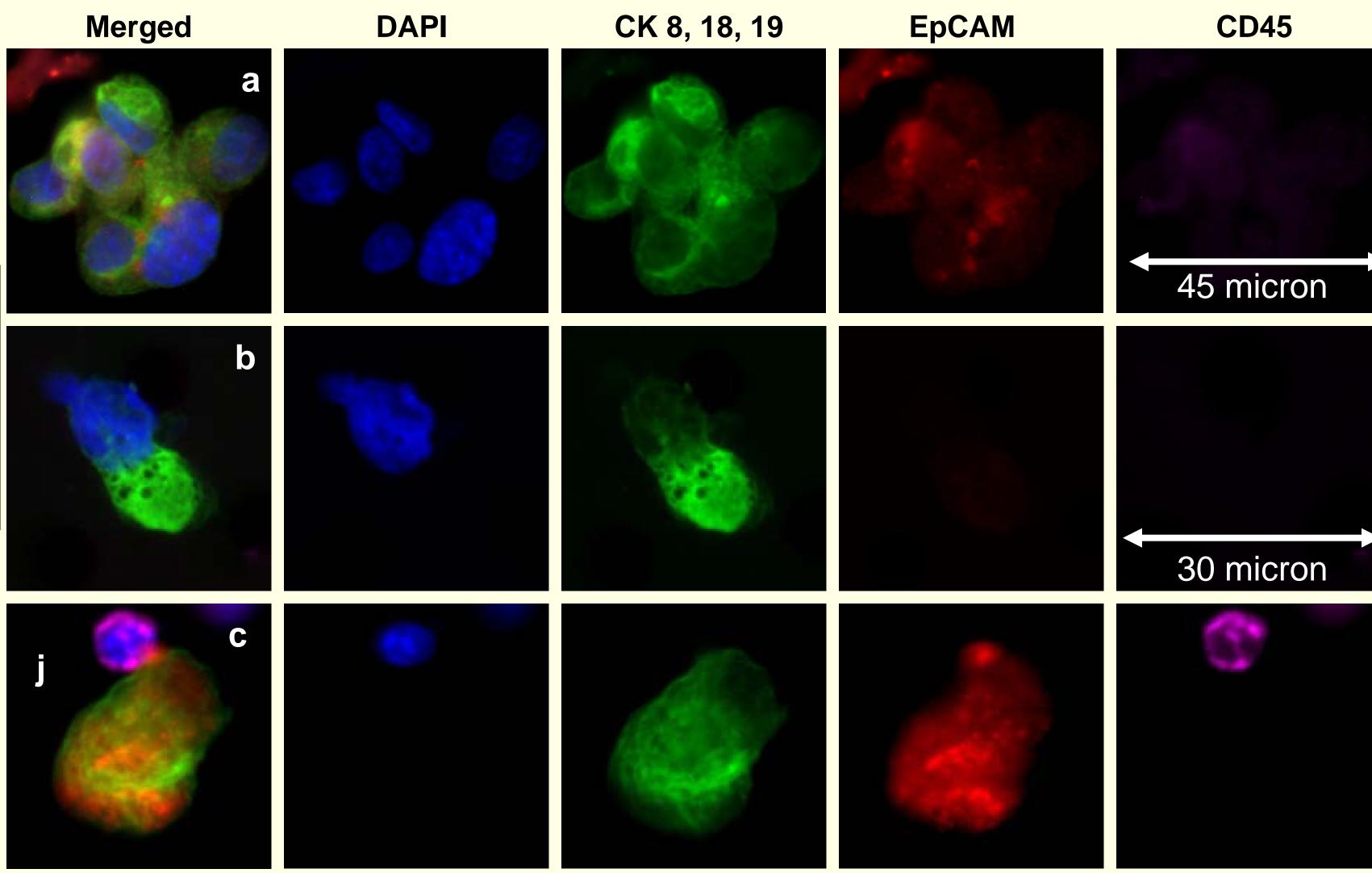


Figure 3. (a) Micrometastasis. (b) Partly extruded CTC. (c) CTC without nucleus next to a white blood cell

## References

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