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cmtang@creatvmicrotech.com
301-983-1650

Characterizing and Correlating CellSieve™ and CellSearch® CTCs by Cytometric Analysis

Daniel L Adams[1], Steingrimur Stefansson[2], Christian Haudenschild[3], Stuart S Martin[4], Monica Charpentier[4], Saranya Chumsri[4], Massimo Cristofanilli[5], Cha-Mei Tang[6], R Katherine Alpaugh[7]

[1]Creatv MicroTech, Inc., Monmouth Junction, NJ, [2]HeMemics Biotechnologies Inc., Rockville, MD, [3]George Washington University, Washington, DC, [4]University of Maryland, Greenebaum Cancer Center, Baltimore, MD, [5]Thomas Jefferson University Hospital, Philadelphia, PA, [6]Creatv MicroTech, Inc., Potomac, MD, [7]Fox Chase Cancer Center, Philadelphia, PA

ABSTRACT

Recent studies reporting hundreds or thousands of circulating tumor cells (CTCs) in the blood of cancer patients have raised questions regarding the prevalence of CTCs, as enumerated by the CellSearch® test. Although CellSearch® detects clinically relevant CTCs, the ability to capture only EpCAM+ cells has led to speculation that it captures only limited subsets of CTCs. In contrast, alternative isolation approaches often capture large numbers of CTCs from patient blood samples and, not surprisingly, these alternative approaches correlate poorly with CellSearch®.

In this method comparison study we compared a microfiltration system (CellSieve™) with CellSearch®, to enumerate CTCs captured from the blood of 30 cancer patients. Like many non-EpCAM techniques, CellSieve™ isolated a greater number of Cytokeratin+ (CK+)/CD45- cells than CellSearch®, and analysis showed a low correlation between the two systems. However, by sub-grouping CK+ cells based on distinct CK staining patterns and nuclear morphologies we elucidated a subpopulation which correlated to CellSearch®. These data suggests that although various CTCs with similar phenotypic expressions are present in cancer patient blood, the clinically relevant cells isolated by CellSearch® can be isolated and identified using a non-EpCAM dependent approach.

INTRODUCTION

CTCs are cells that originate from a primary solid tumor and are found transiting the circulatory system. CTCs have been shown able to monitor therapy response and predict patient outcome.[1-4] Size exclusion is one technique for isolating CTCs irrespective of their surface marker expression capable of isolating various subtypes of CTCs.[2-5]

CellSieve™ microfilters are lithographically fabricated membranes with high porosity, precise pore dimensions, and patterned distribution. We previously reported that CellSieve™ rapidly and efficiently isolates a variety of CTCs from whole peripheral blood, using fluorescent antibody stain as the detection platform. Further, it has been postulated that subtyping by phenotypic determinates may aid identifying the CTCs cellular status for assay comparison, diagnosis, prognosis and therapy determination.[1-5]

MATERIALS & METHODS

Duplicate breast (n=21) and prostate (n=9) patient samples were provided by University of Maryland Greenebaum Cancer Center and Fox Chase Cancer Center (FCCC). CellSieve™ microfiltration used 7.5 mL of whole blood, the same as for CellSearch®, diluted in fixative and filtered through a 7 micron pore microfilter. CTCs collected were stained with DAPI, CK 8, 18 & 19 (FITC), EpCAM (PE), and CD45 (Cy5). CK+/CD45- cells were classified by their CK morphology, nuclear pleomorphism, and presence of EpCAM. Duplicate blood samples were run on CellSearch® following standard protocols at FCCC. Comparative analyses were run on CTC counts by CellSearch® versus the various CTC subtypes captured on CellSieve™.

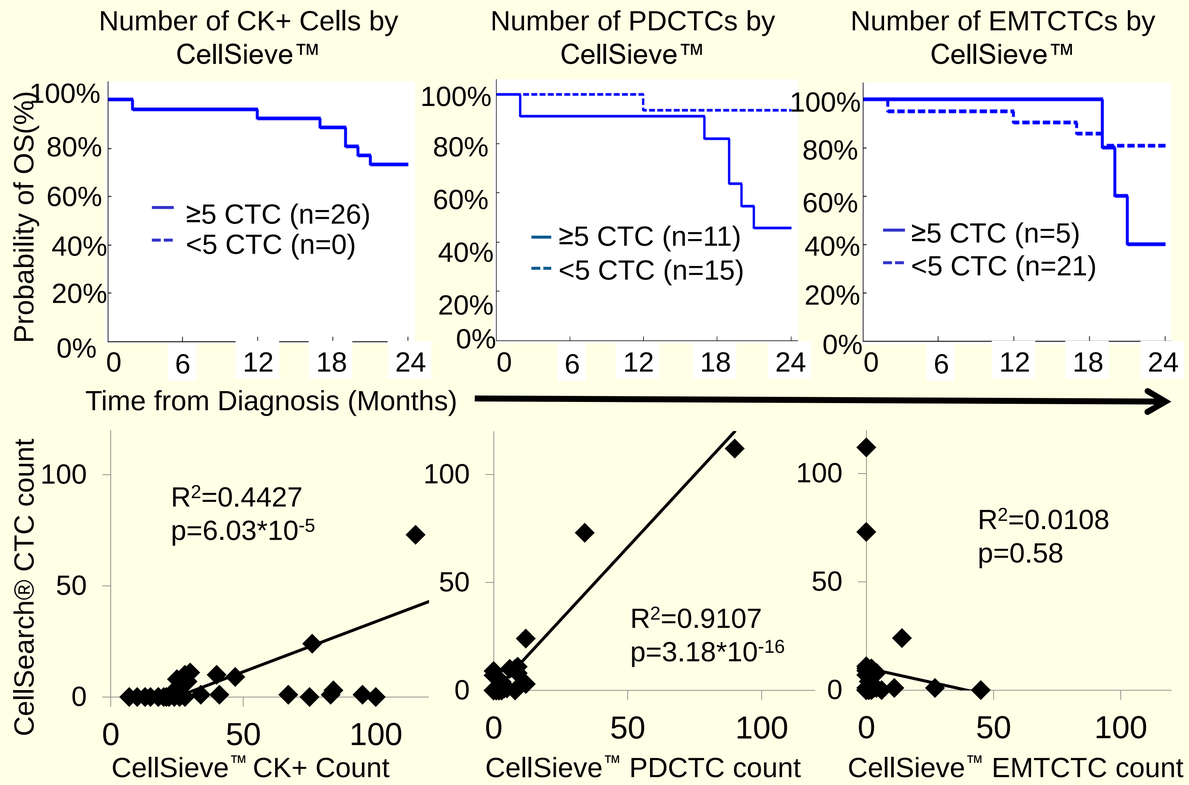

Figure 2. Representative examples of method comparisons and clinical outcomes for three CTC subtypes identified by CellSieve™ versus CTCs enumerated by CellSearch®.

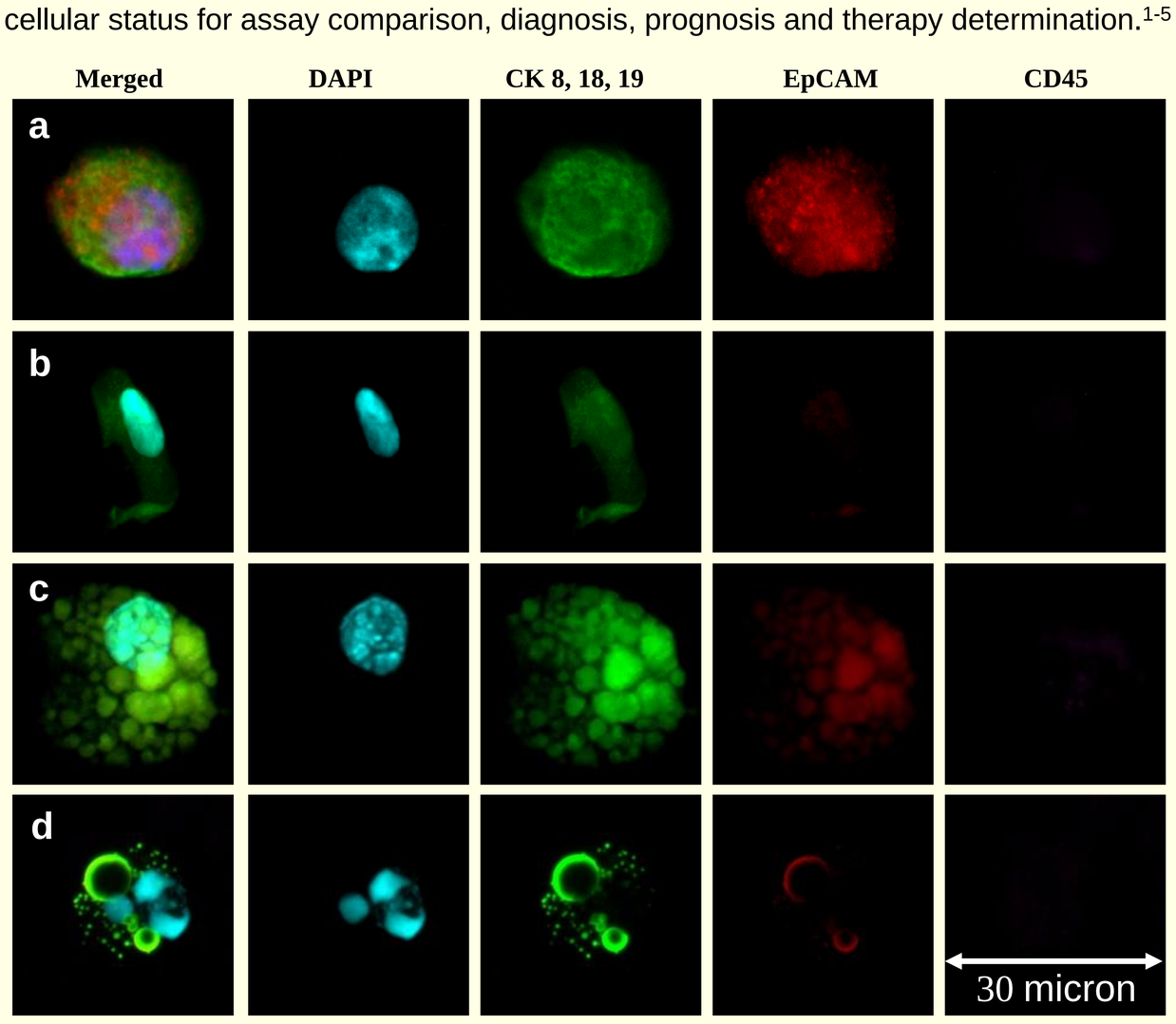

Figure 1. Subpopulations of CTCs from CellSieve™ microfilters. a) CTCs categorized as PDCTCs with filamentous cytokeratin and malignant nuclei. b) EMTCTCs with diffuse cytokeratin patterns and irregular nuclear patterns. c) EACTC with punctate cytokeratin and malignant nuclei d) LACTC with punctate cytokeratin and blebbing nuclei.

RESULTS

- CTCs isolated by CellSieve™ express three distinct cytokeratin, histologically definable, patterns (filamentous, diffuse and punctate).
- CTCs isolated by CellSieve™ can be identified as apoptotic or pleomorphic.
- The total CK+/CD45- CTC group can be subtyped into 4 distinct groups: pathologically definable (PD), epithelial-to-mesenchymal (EMT), early apoptotic (EA) & late apoptotic (LA).
- CK+/CD45- CTCs isolated by CellSieve™ did not correlate to CellSearch® (R^2=0.44, p<0.001).
- PDCTC CTCs with a filamentous CK patterns and pleomorphic nucleus (PDCTC) had a significant correlation with CellSearch® (R^2=0.91, p<0.001).

CONCLUSIONS

- Microfiltration captures CTCs regardless of EpCAM expression.
- Microfiltration captures weakened and apoptotic CTCs.
- A subtype of CK+ CTCs captured by CellSieve™ microfiltration can be statistically correlated with CTCs enumerated by CellSearch®.
- The prognostic implications of CellSearch® CTCs can be applied to non-EpCAM based capture systems.
- The "accepted" clinical cutoff of ≥5 CTCs/7.5 mL for CellSearch® is also a predictor of patient overall survival over a 24 month period using CellSieve™ filtration.

Table 1: Correlations of CK+/CD45- subpopulations identified by CellSieve™ filters versus CellSearch® CTCs

Breast plus Prostate Comparison	Total CK+ cells vs. CellSearch	PDCTC vs. CellSearch	EMTCTC vs. CellSearch	EACTC vs. CellSearch	LACTC vs. CellSearch	Atypical CK+ cells vs. CellSearch	PDCTC+ EpCAM vs. CellSearch	PDCTC+ EACTC vs. CellSearch
R^2	0.4427	0.9107	0.0108	0.6033	0.1311	0.0002	0.94	0.98
p-value	$6.03*10^{-5}$	$3.18*10^{-16}$	0.58	$4.50*10^{-7}$	0.05	0.95	$3.03*10^{-18}$	$6.88*10^{-25}$
Slope	0.45x	1.34x	-0.26x	2.51x	1.40x	0.02x	1.64x	1.07x

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