ApoStream™, a Novel Device for Antibody-independent Capture of Circulating Tumor Cells (CTCs) from Blood of Patients with Various Types of Cancer

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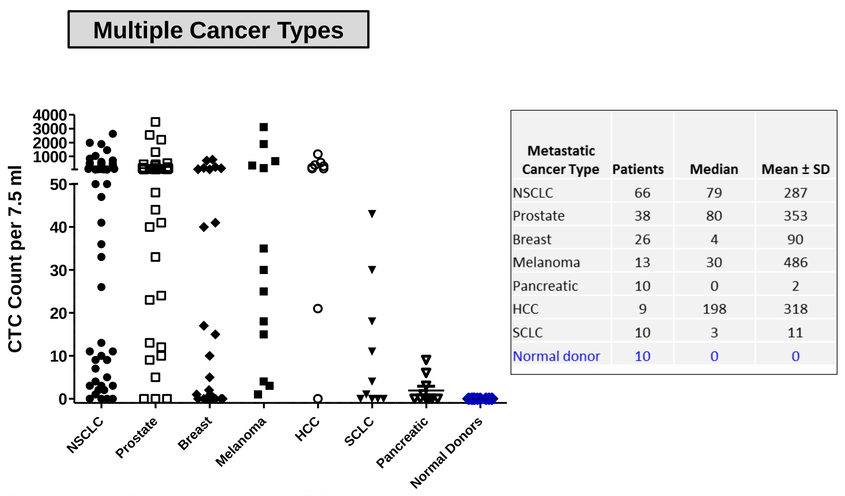
Abstract

Background: Current established methods of circulating tumor cell (CTC) isolation and identification rely on antibodies against epithelial specific markers such as epithelial cell adhesion molecule (EpCAM) and cytokeratin (CK). The classical phenotypic definition of a CTC is a cytokeratin (CK) positive, CD45 negative, nucleated cell, yet several reports have shown that EpCAM and CK detect only a fraction of CTCs and are not sufficient to detect the heterogeneous subpopulations of CTCs. Moreover, subsets of primary tumor cells acquire features of invasiveness and motility and transform into an aggressive phenotype. This process is termed epithelial-mesenchymal transition (EMT) and this altered phenotype is a hallmark of cellular invasion and metastasis. During this process, EpCAM and CK are down regulated or lost leaving a lethal population of CTCs undetectable and unstudied using antibody dependent CTC technologies. It is imperative to isolate CTCs in an EpCAM independent manner and expand the phenotypic characterization of CTCs to elucidate the population heterogeneity and develop context to study the complex biology of CTCs. Here we used ApoStream™, a novel, antibody-independent device which uses dielectrophoresis (DEP) technology in a continuous flow system to isolate and recover CTCs from the blood of cancer patients. In this study, we demonstrate device performance and integration with additional methods to perform subsequent phenotyping and molecular marker analysis.
Methods: The performance of ApoStream™ was assessed using SKOV3 (ovarian cancer) and MDA-MB-231 (breast cancer) cell lines that have a high and low expression level of EpCAM, respectively, to demonstrate linearity and precision of recovery independent of EpCAM receptor levels. Cell viability was assessed by trypan blue dye exclusion assay. ApoStream™ was used to isolate and recover CTCs from prostate, breast, NSCLC, ovarian, hepatocellular, pancreatic, colon and melanoma cancer patient blood. Isolated cells were stained with CK, CD45, and DAPI. CTC enumeration was performed using laser scanning cytometry (LSC). CTC morphology was confirmed with H&E staining. In addition, a multiplexed immunofluorescent assay and LSC analysis were applied to identify multiple combinations of positive and/or negative staining for CK/CD45/DAPI cells, expression of EpCAM and EMT markers. Sequential immunophenotyping and Fluorescence In Situ Hybridization (FISH) analysis were employed to demonstrate feasibility of genetic analysis in CTCs isolated by the ApoStream™ device.
Results: In system precision performance studies, the average recovery of SKOV3 and MDA-MB-231 cancer cells spiked into approximately 12 million peripheral blood mononuclear cells (PBMCs) obtained from 7.5 mL normal human donor blood was 75.4 ± 3.1% (n=12) and 71.2 ± 1.6% (n=6), respectively. The intra-day and inter-day precision coefficients of variation (CVs) of the device were both less than 3%. Linear regression analysis yielded a correlation coefficient (R^2) of more than 0.99 for a spiking range of 4-2600 cells. The viability of MDA-MB-231 cancer cells captured with ApoStream™ was greater than 97.1% and there was no difference in cell growth up to 7 days in culture compared to controls. ApoStream™ recovered varying numbers of CK+/CD45–/DAPI+, CK+/CD45+/DAPI+, CK-/CD45-/DAPI+ cells from each cancer patient sample tested. Additionally, ApoStream™ recovered both EpCAM+ and EpCAM– cells and genetic mutations were identified in isolated CTCs.
Conclusions: The ApoStream™ technology circumvents dependence on expression of EpCAM and recovers CTCs from epithelial and non-epithelial derived tumors. ApoStream™ coupled with LSC or FISH analysis is a sensitive method for phenotyping, genotyping and detecting biomarker expression in CTCs and represents a promising innovative approach to support target-based therapies, facilitate discovery of new therapeutic targets or pursue diagnostics for staging, differentiating and evaluating the disease status in cancer patients.

ApoStream™ CTC Enrichment, Identification and Enumeration



Metastatic Cancer Type	Patients	Median	Mean ± SD
NSCLC	66	79	287
Prostate	38	80	353
Breast	26	4	90
Melanoma	13	30	486
Pancreatic	10	0	2
HCC	9	198	318
SCLC	10	3	11
Normal donor	10	0	0

Figure 1. ApoStream™ isolates CTCs from multiple cancer types.

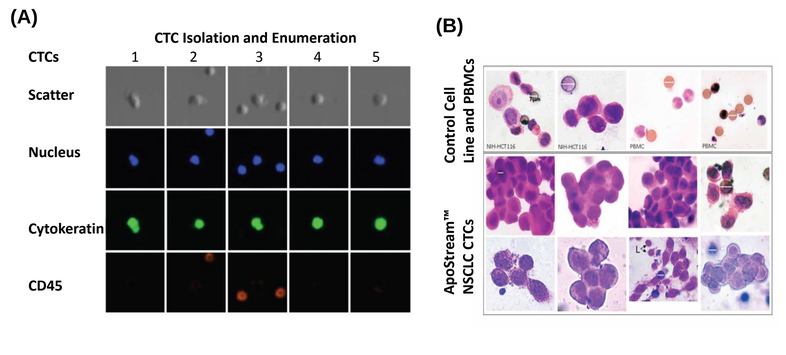
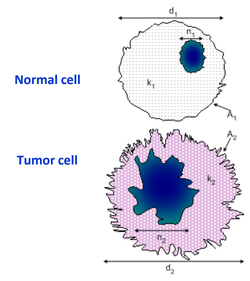


Figure 2. (A) CTCs from NSCLC patients captured by ApoStream™ were identified by immunofluorescent staining using standard DAPI+/CK+/CD45- phenotype.
(B) H&E staining of CTC clusters isolated from the blood of NSCLC patients.

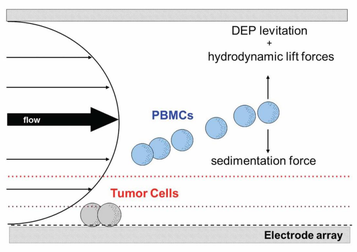
ApoStream™ Technology

(A) Dielectric properties (polarizability) of cells are dependant upon many biophysical features.

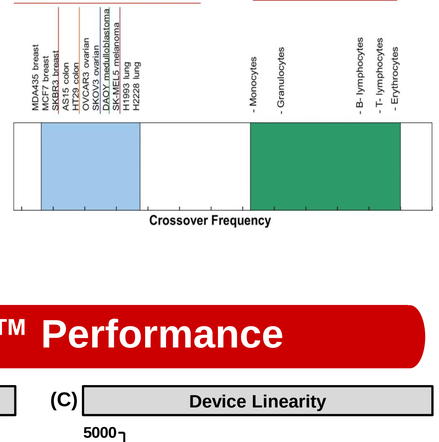
Inherent differences in morphology of CTCs and normal cells result in different polarization charges when exposed to an AC electric current.



(B) Dielectrophoretic, hydrodynamic and sedimentation forces are balanced to attract CTCs and repel normal cells from the chamber floor. CTCs are collected through a port located in the chamber floor while normal cells flow into a waste port.



(C) Cross-over frequencies from different tumor cell types including breast, colon, ovarian, lung and melanoma cell lines and from peripheral blood mononuclear cells (PBMCs) were determined. The differences in cross-over frequencies between cancer and normal cells enable ApoStream™ to separate CTCs from normal cells.



ApoStream™ Isolates CTCs with Multiple Phenotypes and EMT Markers

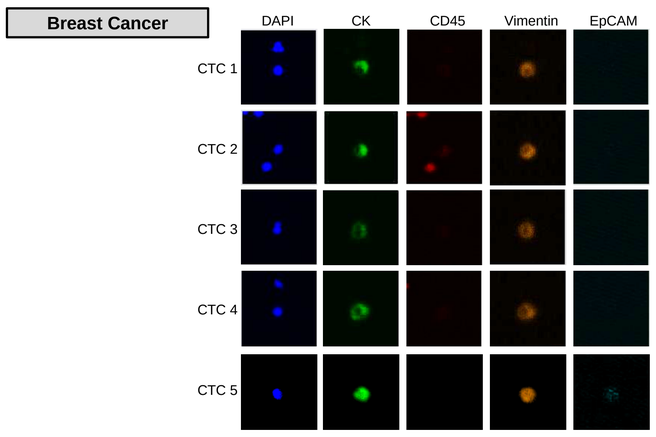


Figure 3. A multiplexed immunofluorescent assay and laser scanning cytometry were used to identify $CK^+/CD45^-/DAPI+$ CTCs and quantify EpCAM and vimentin expression in metastatic breast cancer patients

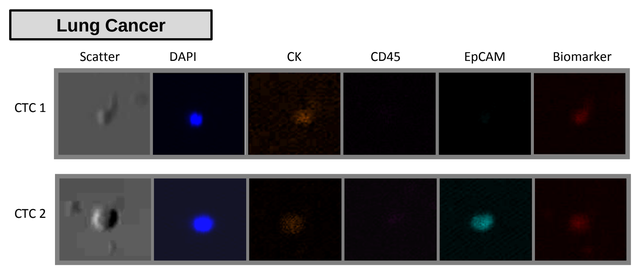


Figure 4. Gallery of representative images of cells isolated by ApoStream™ and stained with antibodies against cytokeratin (CK), CD45, and EpCAM.

Patient ID	Number of $CK^+/CD45^-/DAPI$ cells per 7.5 mL of blood		ApoStream™ ($CK^+/CD45^-/DAPI^+$ cells)			
	CellSearch®	Apostream™	% EpCAM +/ Vimentin -	% EpCAM + / Vimentin +	% EpCAM -/ Vimentin +	% EpCAM -/ Vimentin -
1	0	81	0	3	26	71
2	0	241	0	0	93	8
3	0	40	0	0	100	0
4	0	71	0	11	89	0
5	0	41	0	3	94	3
6	2	149	1	0	83	16
7	0	10	0	0	0	100
8	NA	176	0	0	74	26
9	NA	705	0	0	90	10
10	NA	772	0	0	31	69

Table 1. Distribution of EpCAM/Vimentin phenotypes in $CK^+/CD45^-/DAPI^+$ cells isolated from metastatic breast cancer patient blood by ApoStream™.

Phenotypes	CTC Count from NSCLC patients		
	Patient A	Patient B	Patient C
$CD45^-CK^+$	10	31	52
$CD45^-CK^+EpCAM^+$	0	0	0
$CD45^-CK^+EpCAM^-$	10	31	52

Table 2. ApoStream™ recovered EpCAM negative CK^+ $CD45^-$ CTCs. A larger population of CTCs exist in NSCLC patients that are CK^- and $CD45^-$ (other phenotypes under investigation)

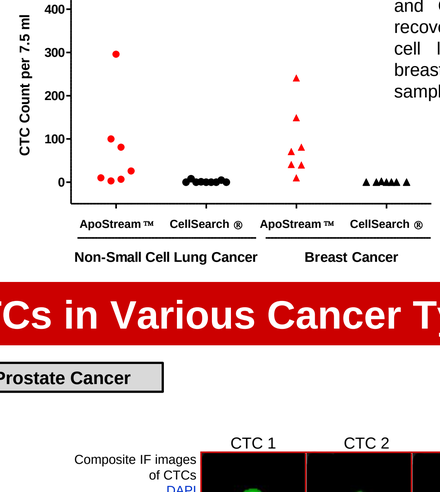
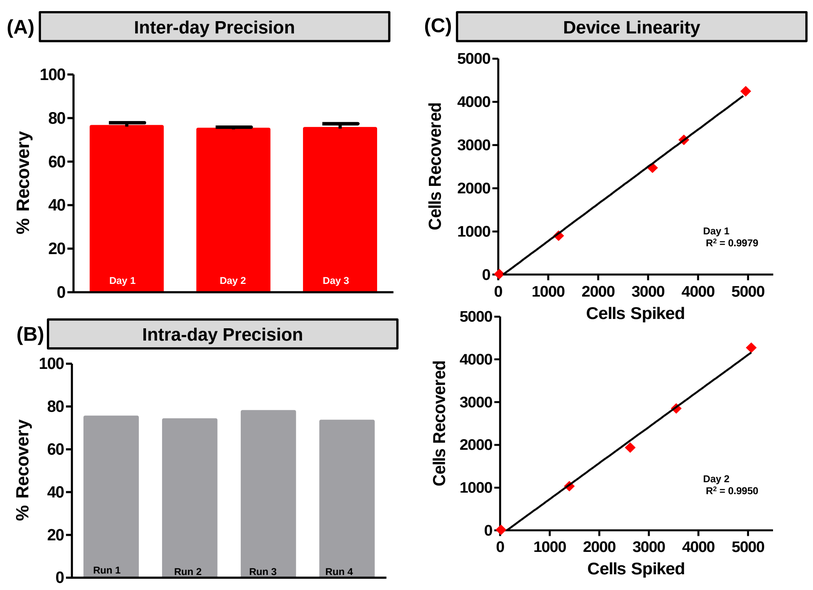


Figure 5. Head to head comparison between ApoStream™ and CellSearch® shows greater recovery of CTCs from non-small cell lung cancer (NSCLC) and breast cancer patient blood samples.

ApoStream™ Performance



(A) Average recovery of SKOV3 cancer cells spiked into PBMCs shows inter-day precision of 75.4 ± 3.1 %, CV = 3.3% (n = 12). (B) Recovery of SKOV3 cancer cells spiked into PBMCs shows intra-day precision of 71.2 ± 1.6 %, CV = 2.7% (n = 6). (C) Device linearity was demonstrated by spiking 4 to ~5000 SKOV3 cells into ~12 million PBMCs from 7.5 mL normal human donor blood.

Biomarker and Genetic Analysis of CTCs in Various Cancer Types

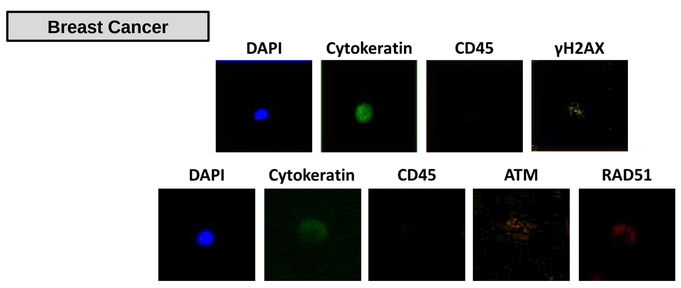


Figure 6. Gallery of representative images of markers of DNA damage on CTCs isolated from breast cancer patients.

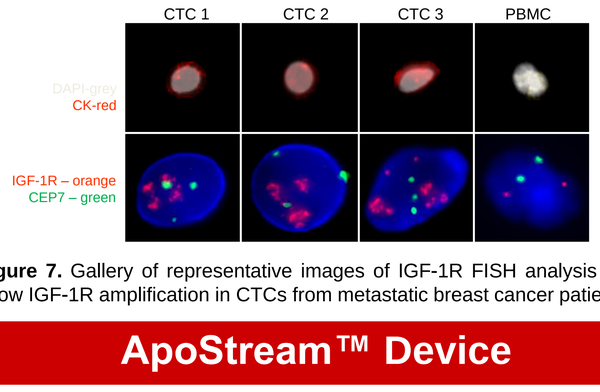


Figure 7. Gallery of representative images of IGF-1R FISH analysis in CTCs show IGF-1R amplification in CTCs from metastatic breast cancer patient.

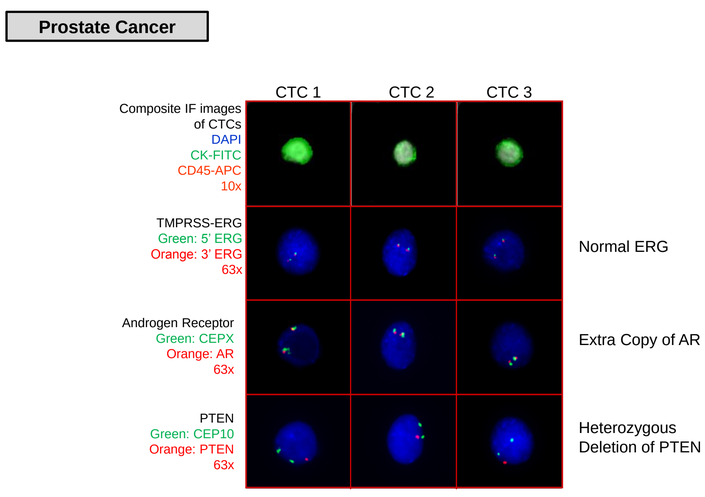
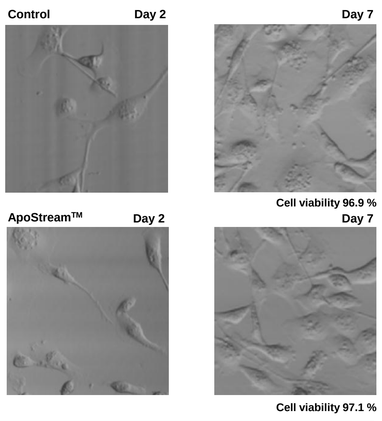
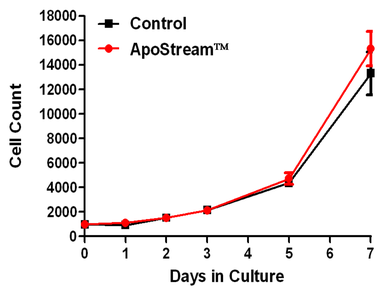


Figure 8. Gallery of representative images of TMPRSS-ERG, Androgen Receptor and PTEN expression in CTCs from prostate cancer patients.

ApoStream™ Preserves Cell Viability



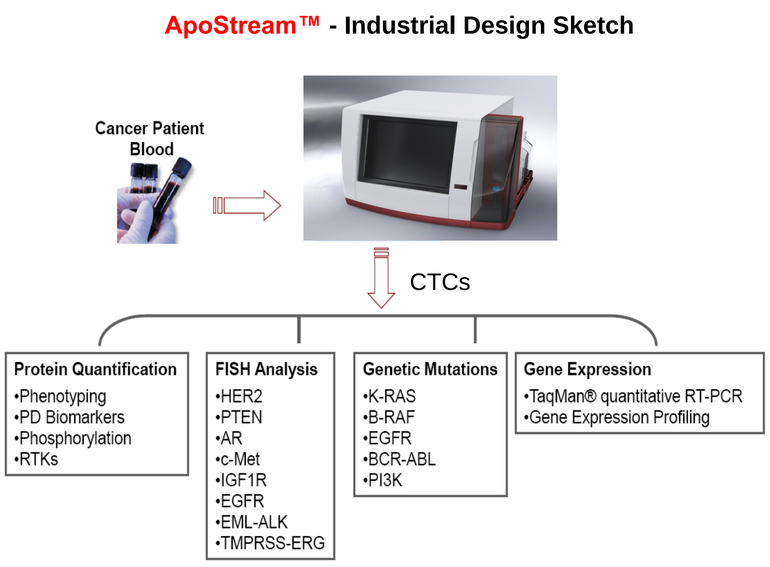
Control and ApoStream™ recovered MDA-MB-231 cancer cells show exponential growth.



Top panel: Images of MDA-MB-231 cell proliferation (Control = no ApoStream™).

Bottom panel: Images of MDA-MB-231 cell proliferation after ApoStream™ separation.

ApoStream™ Device



Summary

- ApoStream™ exploits the differences in biophysical properties between cancer cells and normal cells to isolate and capture CTCs.
- ApoStream™ recovery performance is linear and reproducible.
- ApoStream™ is antibody-independent and isolates CTCs from various types of cancer.
- ApoStream™ recovered higher numbers of CTCs from NSCLC and breast cancer patient blood compared to the EpCAM dependent CellSearch® method.
- ApoStream™ preserves cell viability enabling genetic and molecular analyses.

References & Acknowledgments

1. Vishal Gupta, Insiya Jafferi, Miguel Garza, Vladislava O. Melnikova, David K. Hasegawa, Ronald Pethig and Darren W. Davis. ApoStream™, a new dielectrophoretic device for antibody independent isolation and recovery of viable cancer cells from blood. Biomicrofluidics 6, 024133 (2012).

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