Expanded phenotypic and biological characterization of rare cells isolated from cancer patient blood using ApoStream™

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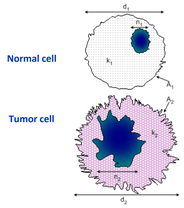
Abstract

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 using current technologies. It is imperative to isolate CTCs in an EpCAM-independent manner, to expand the phenotypic characterization of CTCs to elucidate the population heterogeneity and develop context to study the complex biology of CTCs. It is important to distinguish viable from apoptotic CTCs, as well as cycling and non-cycling CTCs, because these features significantly influence response to therapies. Here we used ApoStream™, a novel antibody-free CTC isolation device to recover circulating rare cells from the blood of cancer patients and perform subsequent phenotyping and molecular marker analysis. **Methods:** Blood samples from breast, prostate, and pancreatic cancer patients were collected and processed using ApoStream™. Isolated cells were stained with CK, CD45, and DAPI. In addition, a multiplexed immunofluorescent assay and laser scanning cytometry analysis were applied to identify multiple combinations of positive and/or negative staining for CK/CD45/DAPI cells, expression of EpCAM and EMT, apoptotic and cell cycle markers.
Results: 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. $Vimentin^+$ cells were detected in several samples suggesting EMT occurs in rare circulating cells. **Conclusions:** ApoStream™ is an ideal CTC detection method that circumvents dependence on expression of EpCAM and recovers CTCs from epithelial and non-epithelial derived tumors. CTCs isolated by ApoStream™ are a source of tissue for expanding the phenotypic definition of CTCs and detecting EMT markers in previously undetected CTCs. Furthermore, examining biological properties within distinct CTC phenotypes may identify subpopulations of cells that are sensitive or resistant to treatment, that may contribute to metastatic progression, or facilitate discovery of new therapeutic targets.

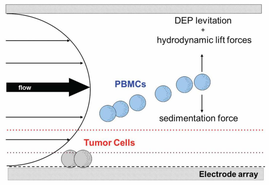
ApoStream™ Technology

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

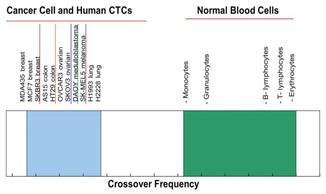
Inherent differences in morphology of CTCs and normal cells result in different polarization charges when exposed to an AC electric current.



(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™ Performance

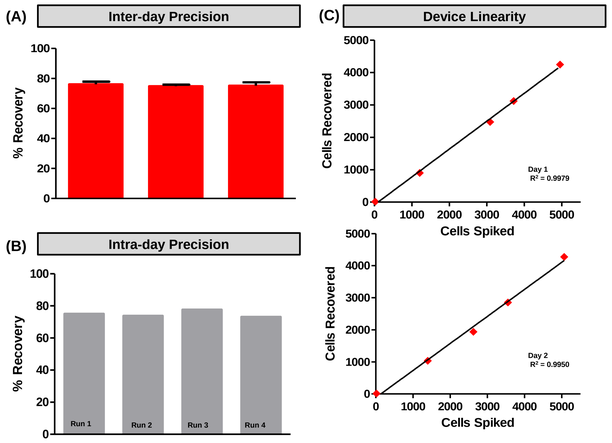


Figure 1. (A) Average recovery of SKOV3 cancer cells spiked into PBMCs shows inter-day precision of 75.4 ± 3.1%, CV = 3.3% (n = 12). (B) Recovery of SKOV3 cancer cells spiked into PBMCs shows intra-day precision of 71.2 ± 1.6 %, CV = 2.7% (n = 6). (C) Device linearity was demonstrated by spiking 4 to ~5000 SKOV3 cells into ~12 million PBMCs from 7.5 mL normal human donor blood.

ApoStream™ Device

ApoStream™ - Industrial Design Sketch

Protein Quantification
- Phenotyping
- PD Biomarkers
- Phosphorylation
- RTKs

FISH Analysis
- HER2
- PTEN
- AR
- c-Met
- IGF1R
- EGFR
- EML-ALK
- TMPRSS-ERG

Genetic Mutations
- K-RAS
- B-RAF
- EGFR
- BCR-ABL
- PI3K

Gene Expression
- TaqMan® quantitative RT-PCR
- Gene Expression Profiling

ApoStream™ CTC Enrichment, Identification and Enumeration

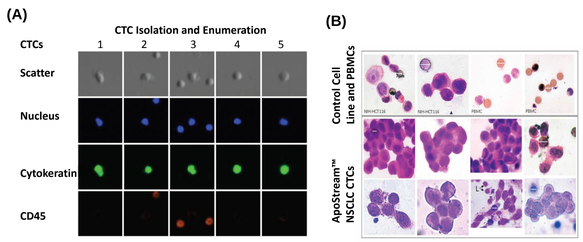
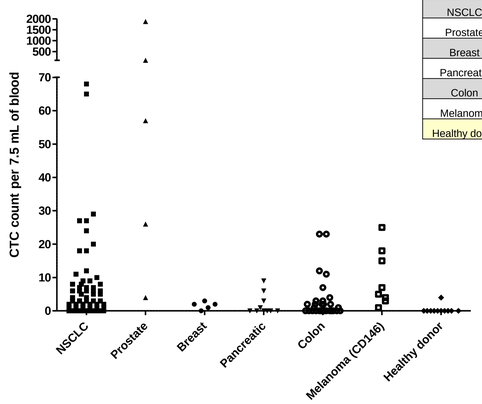


Figure 2. (A) CTCs from NSCLC patients captured by ApoStream™ were identified by immunofluorescent staining using standard DAPI+/CK+/CD45- phenotype.
(B) H&E staining of CTC clusters isolated from the blood of NSCLC patients.

Multiple Cancer Types



Metastatic Cancer Type	Number of Patients	Median CTCs	Mean CTCs
NSCLC	90	1	5
Prostate	5	57	412
Breast	5	2	2
Pancreatic	10	0	2
Colon	29	1	3
Melanoma	8	6	10
Healthy donor	11	0	0

Figure 3. ApoStream™ isolates CTCs from multiple cancer types.

ApoStream™ Isolates CTCs with Multiple Phenotypes and EMT Markers

Breast Cancer

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™. NA-CellSearch® not performed on these samples.

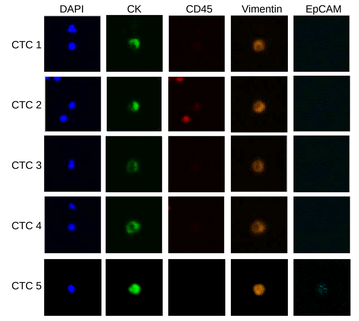


Figure 4. A multiplexed immunofluorescent assay and laser scanning cytometry were used to identify $CK^+/CD45^-/DAPI+$ CTCs and vimentin expression in metastatic breast cancer patients

Prostate Cancer

Patient ID	CellSearch®	ApoStream™
	CK+/CD45- Cell count	CK+/CD45- Cell count
1	19	116
2	0	41
3	0	90
4	1	40
5	1	174
6	1	138
7	41	67
8	11	75
9	8	152
10	21	50

Table 2. Percent and expression of $CK^+/CD45^-$ cells isolated from castrate resistant prostate cancer patient blood by ApoStream™.

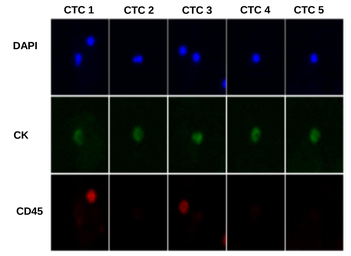


Figure 5. A multiplexed immunofluorescent assay and laser scanning cytometry were used to identify $CK^+/CD45^-/DAPI^+$ CTCs in castrate resistant prostate cancer patients.

Pancreatic Cancer

Patient ID	CellSearch®	Cell phenotypes isolated by ApoStreamTM		
		Cytokeratin phenotypes		
	CK+CD45- cell count	CK+/CD45- cell count	CK-/CD45- cell count	CK+/CD45+ cell count
1	1	9	12	70
2	1	0	20	33
3	0	0	25	28
4	0	0	63	6
5	0	0	16	17
6	0	0	77	76
7	3	3	77	549
8	1	6	166	52
9	10	0	16	7
10	NA	1	83	133

Table 3. Number of cytokeratin positive and negative cells isolated from pancreatic cancer patient blood by ApoStream™. NA-CellSearch® not performed on this sample.

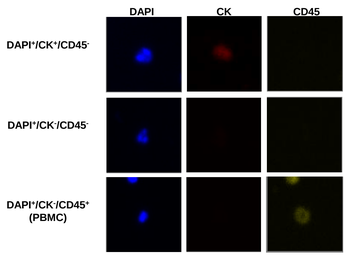


Figure 6. A multiplexed immunofluorescent assay and laser scanning cytometry were used to identify $CK^+/CD45^-/DAPI+$ CTCs in pancreatic cancer patients.

Conclusions & Clinical Significance

- ApoStream™ CTC isolation can be applied to patients of all cancer types, including non-epithelial derived tumors.
- ApoStream™ isolates CTCs from a greater number of patients than currently available technologies.
- Antibody-independent selection used by ApoStream™ allows phenotypic characterization of previously inaccessible CTCs and enables insight into population heterogeneity.
- The increased numbers of CTCs isolated by ApoStream™ enable more robust molecular and genetic analysis to help guide individual treatment decisions.

Reference:
Vishal Gupta, et al. ApoStream™, a new dielectrophoretic device for antibody independent isolation and recovery of viable cancer cells from blood. Biomicrofluidics 6, 024133 (2012).