ApoStream™, a new dielectrophoretic device for antibody-independent isolation and recovery of circulating tumor cells from blood of patients with metastatic pancreatic adenocarcinoma

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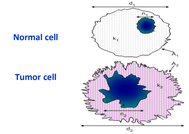
Abstract

Pancreatic adenocarcinoma (PAC) remains the fourth most common cause of cancer-related mortality with a 5-year survival rate of less than 5%. The available diagnostic tools and biomarkers for PAC fail at early detection and suffer from low specificity and sensitivity. Advances in the isolation, recovery, and characterization of circulating tumor cells (CTCs) offer hope for the development of noninvasive techniques for disease detection, monitoring, and biomarker discovery. While CTC enumeration provides prognostic information in patients with various cancer types, the biological characterization of CTCs may offer insight into the molecular determinants of disease progression. Epithelial cell adhesion molecule (EpCAM) and cytokeratin (CK) dependent CTC technologies fare poorly in the metastatic PAC setting, due to altered phenotypes acquired during epithelial mesenchymal transition (EMT). The links between EMT, plectin-1, mesothelin and metastatic progression of PAC are emerging and underscore the need for biomarker information in real time. Here we used ApoStream™, a novel, antibody-independent device which uses dielectrophoretic technology in a continuous flow system to isolate CTCs from the blood of patients with metastatic PAC and expand their phenotypic characterization in order to elucidate the population heterogeneity and characterize pancreatic specific markers (CA19-9, *KRAS*, plectin-1 and mesothelin). This prospective study will evaluate thirty patients. Paired blood samples from 11 metastatic PAC patients were analyzed by CellSearch® and ApoStream™. Collected cells were immunostained using antibodies against CK, CD45, DAPI, CA19-9, plectin-1 and mesothelin. CTC enumeration was performed using laser scanning cytometry (LSC). A multiplexed immunofluorescent assay and LSC analysis were applied to identify cell phenotypes based on combinations of CK, CD45, plectin-1 and mesothelin marker expression. **Results:** The detection of CK$^+$/CD45$^-$/DAPI$^+$ cells was comparable between CellSearch® and ApoStream™ with counts ranging from 1-10 CTCs/7.5 mL blood in 50% of patients. In addition, ApoStream™, recovered CK$^-$/CD45$^-$/DAPI$^+$ cells in 100% of patients with counts in the range of 12-166 cells/7.5 mL of blood. CA19-9$^+$ cells were identified in both CK$^+$/CD45$^-$/DAPI$^+$ and CK$^-$/CD45$^-$/DAPI$^+$ subpopulations isolated by ApoStream™. *KRAS*, plectin-1 and mesothelin analysis is pending. **Conclusions:** ApoStream™ recovers putative CTCs with multiple phenotypes in patients with metastatic PAC. Preliminary data is encouraging and if confirmed in a larger sample size of PAC patients, ApoStream™ could prove to be a sensitive method for isolating and detecting biomarkers in CTCs of PAC patients. **Acknowledgments:** Supported in part by the Lockton Fund.

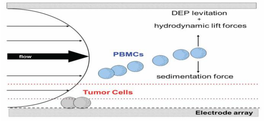
ApoStream™ Technology

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

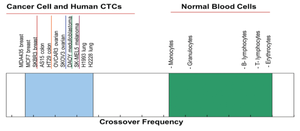
Inherent differences in morphology of CTCs and normal cells result in different polarization charges when exposed to an AC electric current.



(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

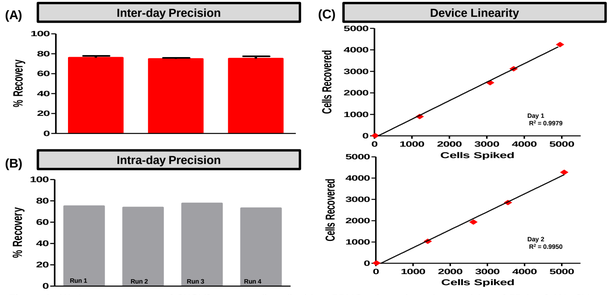


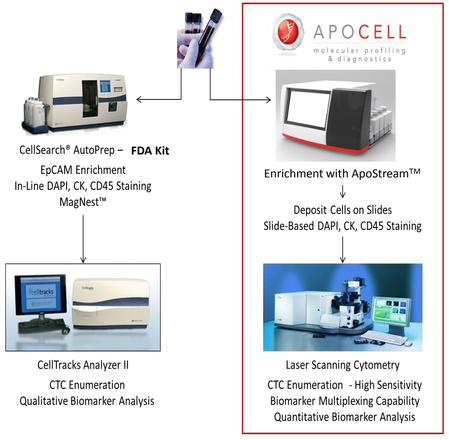
Figure 1. (A) Average recovery of SKOV3 cancer cells spiked into PBMCs shows inter-day precision of 75.4 ± 3.1%, CV = 3.3% (n = 12). (B) Recovery of SKOV3 cancer cells spiked into PBMCs shows intra-day precision of 71.2 ± 1.6 %, CV = 2.7% (n = 6). (C) Device linearity was demonstrated by spiking 4 to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

ApoStream™ Prototype Device

ApoStream™ - Prototype

Current prototype design shipped to National Cancer Institute & Massey Cancer Center, VA, in Dec 2012

Methods



Pancreatic Cancer Cell Line Recovery with ApoStream™

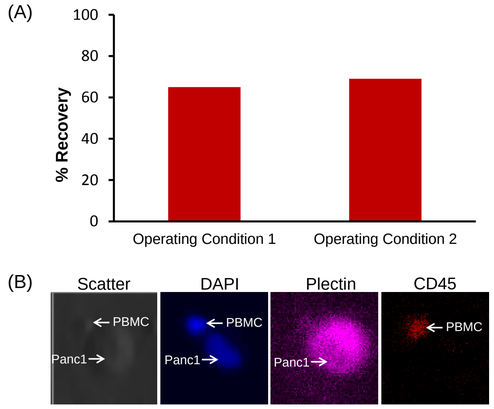


Figure 2. (A) Recovery of Panc1 cancer cells spiked into healthy donor PBMCs was 65% using DEP Operating Condition 1 and 69% using DEP Operating Condition 2. (B) ApoStream™ enriched cells were immunostained with antibodies against Plectin and CD45. Plectin staining was found to be specific to Panc1 cells and not healthy donor PBMCs.

Side-by-Side Comparison of Pancreatic CTC Isolation with Apostream™ vs CellSearch®

Patient #	CellSearch®	ApoStream™			
		Cytokeratin phenotypes		CA 19.9 phenotypes	
	CK$^+$/CD45$^-$ cell count	CK$^+$/CD45$^-$ cell count	CK$^-$/CD45$^-$ cell count	CA 19.9$^+$/ CK$^+$/CD45$^-$ cell count	CA 19.9$^+$/ CK$^-$/CD45$^-$ cell count
1	1	9	12	5	0
2	1	0	20	0	0
3	0	0	25	0	0
4	0	0	63	0	1
5	0	0	16	0	0
6	0	0	77	0	31
7	3	3	77	1	1
8	1	6	166	0	0
9	10	0	16	0	1
10	NA*	1	83	1	1

NA*, not available; sample was aborted by CellSearch® due to sample rejection

Table 1

- ApoStream™ isolated an equal or greater number of CK+/CD45- cells compared to the CellSearch® platform in 3 of 5 pancreatic cancer patient samples with detectable CK$^+$/CD45$^-$ cells.
- Both systems did not detect CK$^+$/CD45- cells in the other 4 patient samples
- Further investigation is required to understand the significance of cells with CK-/CD45- and CK+/CD45+ phenotypes

Representative Images of Cells Isolated by ApoStream™ from Patient 1

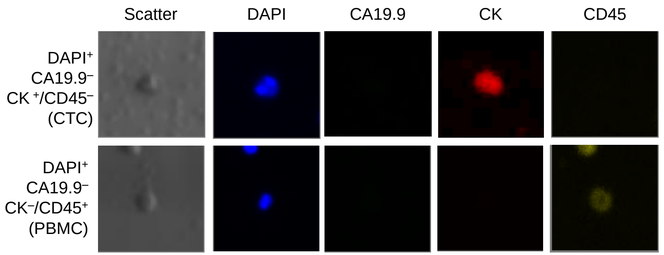


Figure 3. CTCs were isolated from blood of a pancreatic cancer patient using ApoStream™. CTCs were immunostained with antibodies against CA19-9, CK and CD45. Representative images identify candidate CTC (CK$^+$/CD45$^-$) and a normal PBMC cell (CK$^-$/CD45$^+$).

Frequency of CTC-Positive Pancreatic Cancer Patients

No. of Patients	Mean CTC Number per 7.5 mL of Blood	Median CTC Number per 7.5 mL of Blood	Range of CTC Numbers per 7.5 mL of Blood	% Patients with CTC >0
18	2	2	0 – 9	61

Summary & Clinical Significance

- ApoStream™ CTC isolation can be applied to all cancer types, including non-epithelial derived tumors because the basis for isolation is independent of antibodies to cell surface antigens (EpCAM).
- Antibody-independent selection used by ApoStream™ allows phenotypic characterization of previously inaccessible CTCs and enables insight into CTC population heterogeneity.
- Plectin-1 is a specific biomarker for pancreatic cancer[2] while CA 19.9 is less specific.
- Inclusion of specific tumor associated markers like plectin will enable the expansion of the classical phenotypic definition of CTCs and monitoring of PAC patients.

References:

[1]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).

[2]Dirk Bausch, et al. Plectin-1 as a novel biomarker for pancreatic cancer. Clin Cancer Res 12(2);2011.