Molecular characterization of circulating tumor cells recovered from metastatic pancreatic cancer patients using ApoStream™, a new antibody-independent dielectrophoretic device

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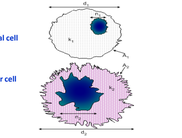
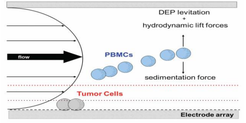
Abstract

Background: Pancreatic adenocarcinoma (PAC) remains the fourth most common cause of cancer-related mortality due to late diagnosis and limited treatment options. The available diagnostic tools and biomarkers for PAC fail at early detection and suffer from low sensitivity and specificity. Advances in the recovery and characterization of circulating tumor cells (CTCs) offer hope for the development of noninvasive techniques for earlier disease detection, monitoring response to therapy, and identification of druggable targets and biomarkers. 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 and sensitivities or resistance to treatment regimens. 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, KRAS, plectin-1, mesothelin and metastatic progression of PAC are emerging and underscore the need for biomarker information in real time. **Material and methods:** We used ApoStream™, a novel, antibody-independent device which uses dielectrophoretic technology in a continuous flow system to isolate CTCs[1] from the blood of metastatic PAC patients and expand their phenotypic identities to elucidate 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 10 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. A multiplexed immunofluorescent assay and laser scanning cytometry (LSC) analysis were applied to enumerate CTCs and 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 on CTCs will be presented. **Conclusions:** ApoStream™ recovered classical and 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™ combined with molecular characterization could prove to be a sensitive method for isolating and detecting biomarkers in CTCs of PAC patients. **Acknowledgements:** The Lockton Fund and NCI Contract No. HHSN261200800001E.

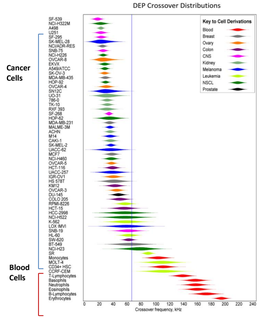
ApoStream™ Technology



(A) Dielectric properties (polarizability) of cells are dependent upon cell diameter, membrane morphology and conductivity. Inherent differences in morphology of CTCs and normal cells result in different dielectric polarization charges when exposed to an AC electric current.



(B) Dielectrophoretic, hydrodynamic and sedimentation forces are utilized 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.[2] The differences in cross-over frequencies between cancer and normal cells enable ApoStream™ to separate CTCs from normal cells.

ApoStream™ Performance

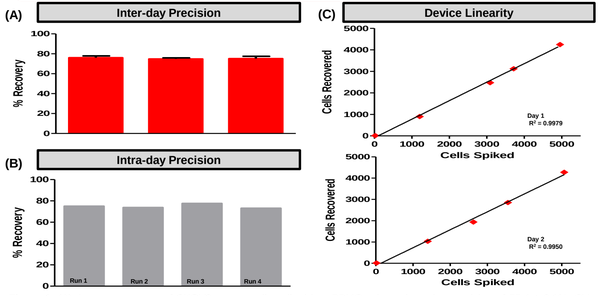
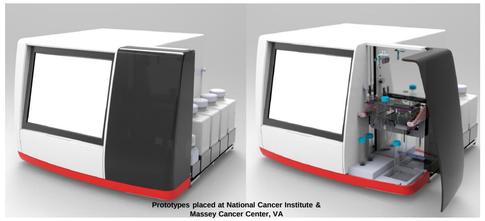


Figure 1. (A) Average recovery of SKOV3 cancer cells spiked into PBMCs shows inter-day precision of 75.4 ± 3.1%, CV = 3.3% (n = 12), (B) Recovery of SKOV3 cancer cells spiked into PBMCs shows intra-day precision of 71.2 ± 1.6 %, CV = 2.7% (n = 6). (C) Device linearity was demonstrated by spiking 4 to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

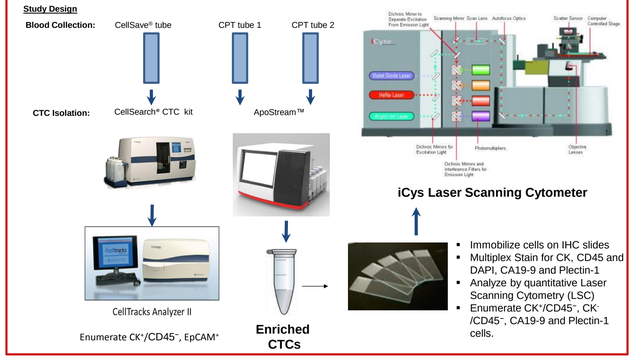
ApoStream™ Prototype Device



Prototypes placed at National Cancer Institute & Massey Cancer Center, VA

Methods

All patients provided signed, written informed consent for this laboratory-based research study approved by UTMDACC IRB.



Pancreatic Cancer Cell Line Recovery with ApoStream™

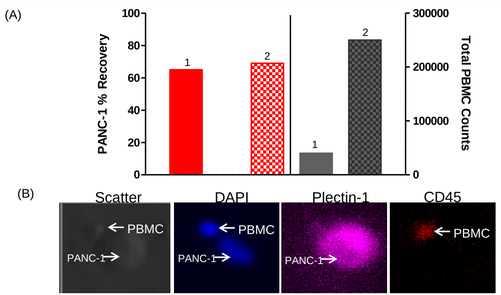


Figure 2. (A) Recovery of PANC-1 cancer cells spiked into healthy donor PBMCs was 65% using DEP Operating Condition 1 and 69% using DEP Operating Condition 2. (B) ApoStream™ enriched cells were immunostained with antibodies against plectin-1 and CD45. Plectin-1 staining was found to be specific to PANC-1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

CTC Isolation with CellSearch® vs Apostream™

Table 1

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®

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. Neither system detected CK$^+$/CD45$^-$ cells in 4 patient samples. Further investigation is underway to understand the significance of cells with CK$^-$/CD45$^-$ phenotypes.

Heterogeneous CTC Phenotypes in PAC Patients

Table 2

Patient #	Cytokeratin Phenotypes		CA19-9 Phenotypes		Plectin-1 Phenotypes	
	CK+/CD45-	CK-/CD45-	CA19-9+/ CK+/CD45-	CA19-9+/ CK-/CD45-	Plectin-1+/ CK+/CD45-	Plectin-1+/ CK-/CD45-
14	19	213	0	8	1	0
15	2	2	0	1	0	0
16	3	668	1	55	1	11
17	14	358	11	364	14	15
18	9	121	0	7	0	1
19	4	111	0	3	3	5
20	0	0	0	0	0	0
21	2	8	0	1	0	0
22	0	104	0	55	0	0
23	1	16	0	1	0	0

*Results are the average of duplicate blood samples

CK$^+$/CD45$^-$ cells were detected in 80% of pancreatic cancer patient blood samples. CK$^+$/CD45$^-$CA19-9$^+$ cell phenotypes were detected in 20% patients and CK$^+$/CD45$^-$ plectin-1$^+$ cell phenotypes were detected in 40% patients. Patients with CK$^+$/CD45$^-$ CA19-9$^+$ phenotype co-expressed plectin-1.

CK$^-$/CD45$^-$ cell phenotypes were detected in 90% of pancreatic cancer patient blood samples. CA19-9$^+$ expression was detected in 90% of samples and plectin-1 expression was detected in 40% of samples.

Representative Images of CTCs Isolated by ApoStream™

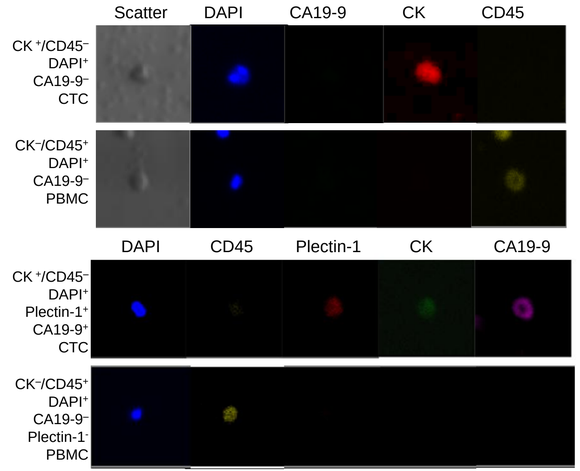


Figure 3. Representative images identify classical CTCs (CK$^+$/CD45$^-$) with associated pancreatic cancer markers CA19-9 and plectin-1 and normal PBMC (CK$^-$/CD45$^+$).

Summary & Clinical Significance

- Antibody-independent rare cell isolation by ApoStream™ combined with phenotypic characterization allows identification of previously undetectable CTCs and enables insight into CTC population heterogeneity.
- Plectin-1 has been shown to be a specific biomarker for pancreatic cancer[3] while the specificity of CA19-9[4] as a pancreatic CTC biomarker will require further investigation.
- Mesothelin was evaluated as a biomarker for pancreatic CTCs. Low antibody signal to background ratio and lack of specificity suggest it is not fit for this purpose.
- Inclusion of potential tumor associated markers like CA19-9 and plectin-1 may enable the expansion of the classical phenotypic definition of CTCs and monitoring of PAC patients.
- 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 like EpCAM.

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]Sangjo Shim et al. Dielectrophoresis has broad applicability to marker-free isolation of tumor cells from blood by microfluidic systems. Biomicrofluidics, 7, 011808, 2013.

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

[4]Chuanli Ren, et al. Detection of apoptotic circulating tumor cells in advanced pancreatic cancer following 5-FU chemotherapy. Cancer Biology and Therapy, 12(8);2011.