ApoStream™, an Antibody Independent Platform, Captures Circulating Tumor Cells in Patients with Hepatocellular Carcinoma

Andrew Poklepovic[1], John D. Roberts[1], Maria Quigley[1], Vladislava Melnikova[2], Jacky Woo[2], Weigou Wu[2], Kenna Anderes[2], Darren Davis[2].
[1]VCU Massey Cancer Center, Richmond, VA; [2]ApoCell, Inc, Houston, TX

Abstract #2381

Background

Biopsies of HCC can be technically difficult, given the vascular nature of the liver and underlying liver disease. Many HCC patients do not get biopsies, and molecular characterization of these tumors is not possible. Capture of CTCs from blood allows for analysis of cancer cells in metastatic dissemination. The use of EpCAM-based enrichment platforms limits the type of tumor cells that can be recovered, as it selects only for cells which express the antigen of interest. Nonselective methods of CTC analysis are needed. An ongoing study is evaluating the recovery of CTCs in HCC patients with elevated serum alpha-fetoprotein (AFP) using the novel antibody-independent ApoStream™ platform, which utilizes the principle of dielectrophoretic field-flow fractionation to position cells in a hydrodynamic flow profile for sorting.

Methods

Paired 7.5 ml blood samples from HCC patients were analyzed by CellSearch™ and ApoStream™. Collected cells were immunophenotyped using antibodies against Cytokeratin (CK), CD45, DAPI, and AFP, and enumerated by quantitative laser scanning cytometry.

Biophysical Basis for Separation of CTCs

A) In response to AC electrical field stimulation, cells are attracted to, or repulsed from, the source of that field. The frequency at which the cell shifts from attraction to repulsion is known as the crossover frequency. The crossover frequency of cancer cells is different from peripheral blood mononuclear cells, and allows for tumor cells to be attracted to the electrical plate while normal cells are repulsed into the center of the flow chamber.

B) Dielectrophoretic, hydrodynamic, and sedimentation forces are balanced to attract CTCs to, and repel normal cells from, the chamber floor. PBMCs are positioned in the center of the flow column, and move through the chamber quickly into a waste port. CTCs are attracted to the electrical plate, move more slowly along the flow column, and are collected through a port located in the chamber floor. Cells remain viable after collection.

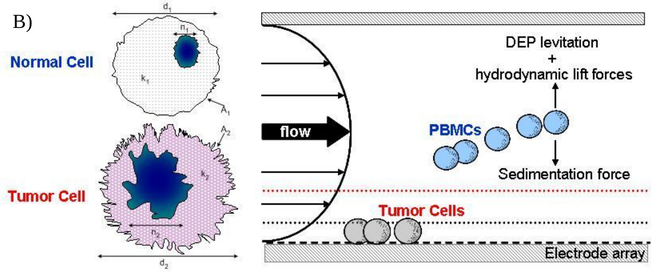
CTCs Isolated from HCC Patients

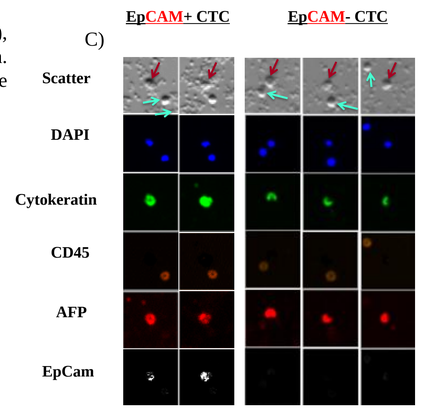
Patient #	Subject ID	Serum AFP level ng/mL	Macrovascular Invasion	Extrahepatic Disease	Therapy at time of sample collection	DAPI⁺CD45⁻CK⁺ (Typical CTCs)							DAPI⁺CD45⁻CK⁻AFP⁺ (ApoStream™)	DAPI⁺CD45⁺CK⁺ (ApoStream™)		
						CTC count by CellSearch®	CTC Count by ApoStream™									
							Total	AFP MFI	AFP⁺	AFP⁻	EpCAM+	EpCAM-		Total	CD45+/CK+ /AFP+	CD45+/CK+ /AFP-
1	1-L-01	36995	Yes	Abdominal LN	Sorafenib 400mg BID	0	0	NA	0	0	NA*	NA*	14	1	1	0
2	1-L-02	728	No	No	Sorafenib 400mg BID	1	21	512,033	13 (62%)	8	NA*	NA*	9	10	9	1
3	1-L-03	78	Yes	No	Sorafenib 400mg BID	0	125	1,841,482	90 (72%)	35	NA*	NA*	85	235	184	51
4	1-L-04	60	No	Prior Tumor Rupture	None	0	554	3,600,799	540 (97%)	14	NA*	NA*	17	1047	978	69
5	1-L-05	2278	Yes	No	Sorafenib 200mg bid	0	1165	3,806,105	1049 (90%)	157	NA*	NA*	1	1902	622	1280
6	1-L-06	3049	Yes	Abdominal Wall Implants	Sorafenib 400mg BID	0	29	0	0	29	NA*	NA*	0	203	0	203
7	1-L-07	31522	Yes	Abdominal LN	None	0	380	506,989	376 (99%)	4	NA*	NA*	0	1418	367	1051
8	1-L-08	29	No	No	None	0	198	0	0	198	NA*	NA*	0	42	0	42
9	1-L-09	4083	No	No	None	0	121	1,300,207	52 (43%)	69	NA*	NA*	0	131	23	108
10	1-L-10	19219	Yes	Abdominal LN	None	0	803	2,164,083	746 (93%)	57	87 (11%)	716	0	3755	3472	283

Results

Results from the 10 patients in this pilot project are listed in the table above. Serum AFP at time of collection, the presence of portal or hepatic vein invasion by tumor, presence of metastases, and therapy at time of CTC analysis are listed. In comparison with the CellSearch® platform, ApoStream™ isolated a higher number of CK+/CD45-/DAPI+ CTCs in HCC cancer patients. Nine out of ten (90%) of patients had detectable CTCs using the typical definition (21-1165 cells). Within most individual patients, AFP(+) and AFP(-) CTCs were collected, demonstrating tumor heterogeneity. ApoStream™ also isolated potential CTCs with the AFP+/CK-/CD45-/DAPI+ phenotype (1-85 cells), and cells with AFP+/CK+/CD45+/DAPI+ phenotype (1-3472 cells). ApoStream™ detected EpCAM (+) and EpCAM (-) cells within the same patient.
NA*, EpCAM measurement not performed.

C) Fluorescent imaging of CTCs, from patient #10. AFP+ CTCs (red arrows), demonstrate CK+/CD45-/DAPI+ phenotype, and variable EpCAM expression. PBMCs (turquoise arrows) demonstrate CK-/CD45+/DAPI+ phenotype, and are EpCAM negative.





Conclusions

- The ApoStream™ system successfully recovers CTCs from HCC patients.
- ApoStream™ captures more CTCs from HCC patients than CellSearch®.
- ApoStream™ isolates CTCs with multiple phenotypes within the same patient.
  - AFP(+) and AFP(–) CTCs
  - EpCAM (+) and EpCAM (–) CTCs
- ApoStream™ is well suited to advance clinical research in HCC patients.

Future Directions

ApoStream™ CTC analysis is being used for pharmacodynamic analysis in an ongoing phase I study of the combination of sorafenib and vorinostat in patients with advanced HCC. In preclinical models, the combination synergistically induces tumor cell death through activation of CD95. In this study, CTC analysis will compare the expression of CD95 prior to and after drug combination exposure. ApoStream ™ is also being used for CTC analysis in other tumor types.