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

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Abstract

Background: Cancer detection requires the identification and quantification of circulating tumor cells (CTCs) isolated from the peripheral blood of cancer patients. This is crucial for a variety of cancer types and treatments. Current methods include multiple technologies, each with advantages and limitations. A single isolation platform should be able to isolate a wider range of CTCs and provide a detailed analysis of their characteristic properties.

Methods: We utilized ApoStream™, a single-platform technology that enables the isolation of CTCs in a process described. CTCs were isolated from peripheral blood by density gradient centrifugation followed by immunomagnetic labeling. We stained CTCs using anti-EpCAM, anti-Cytokeratin-4, and anti-CD45 antibodies, and analyzed their morphology and intracellular keratin content using immunofluorescent stain.

Results: We isolated CTCs from peripheral blood and observed them under microscope to confirm their presence. We determined that CTCs were easily isolated from the blood of cancer patients and morphologically characterized.

Conclusion: ApoStream™ technology provides a single-platform isolation and phenotypic characterization of CTCs, thereby advancing cancer detection and treatment strategies.

ApoStream™ Technology

(A) Dielectric properties (polarizability) of cells are dependent upon many physical biological features. Inherent differences in morphology of CTCs and normal cells result in different polarization changes 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) Cytokine levels from different tumor cell types, such as breast, colon, ovarian, lung, and melanoma cell lines, and from peripheral blood mononuclear cells (PBMCs) were determined. The differences in cytokine levels reflect the cancer and normal cells, which enable ApoStream™ to separate CTCs from normal cells.

ApoStream™ Device

ApoStream™ + Industrial Design Sketch

CTC Isolation and Enumeration

Pancreatic CTCs

Multiple Cancer Types

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.

References:

ApoStream™ Isolates CTCs with Multiple Phenotypes and EMT Markers

Table 1. Distribution of EpCAM/Vimentin phenotypes in C/CD45/DAPI+ CTCs isolated from metastatic breast cancer patient blood by ApoStream™. NA-CellSearch® not performed on these samples.

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

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 1. A multiplexed immunofluorescent assay and laser scanning cytometry were used to identify C/CD45-DAPI+ CTCs in castrate resistant prostate cancer patients.

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

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

Figure 4. A multiplexed immunofluorescent assay and laser scanning cytometry were used to identify C/CD45-DAPI+ CTCs in castrate resistant prostate cancer patients.

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