Abstract # 3062

Isolation and Characterization of Circulating Tumor Cells (CTCs) from Peripheral Blood Specimens of Patients with Advanced Solid Tumor Malignancies (Using ApoStream® Instrumentation)

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Introduction

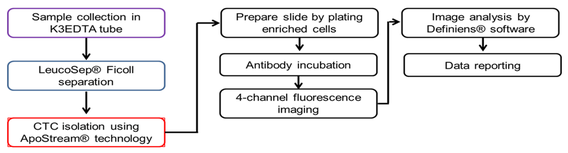
Circulating tumor cells (CTCs) isolated using an antibody-dependent capture method have been shown to be a strong independent prognostic factor for progression-free and overall survival in certain carcinomas. However, the applicability of this technology is limited to epithelial malignancies. Utility may be even further restricted by epithelial mesenchymal transition (EMT) associated with chemotherapy and targeted agent therapy. We have reported previously that patients in our clinic, who have typically failed at least 3 lines of therapy are generally negative for CTCs captured by the EpCAM method [1, and manuscripts in preparation].

An antibody-independent methodology, ApoStream®, is capable of isolating live CTCs from epithelial and non-epithelial malignancies by exploiting the morphological and biophysical differences between cancer cells and normal blood cells. Viable cells are essential for isolation using ApoStream® technology, and patient cancer diagnosis information is critical to determine system operating parameters.

An initial clinical readiness study is being conducted using specimens from patients with advanced sarcomas and carcinomas.

Methods

Blood specimens from patients enrolled in clinical trials at the National Cancer Institute were collected in BD CPT tubes, K3EDTA tubes, or ACD tubes and processed on the same day. PBMC fractions were isolated following the manufacturer's recommended protocol for CPT tubes or by LeucoSep® separation [2]. PBMC pellets were resuspended in ApoStream® sample buffer and run through the instrument at predetermined operating conditions. The enriched fraction was spun down, immediately plated onto Marienfeld® slides, fixed, and stored at 4°C until further processing. All patients gave written informed consent and were enrolled on NCI Institutional Review Board (IRB)–approved protocols.



Target	Clone	Vendor	Fluorochrome
Mucin-1 (MUC1)	E29	SCBT	Unconjugated
Carcinoembryonic antigen (CEA)	II-7	DAKO	Unconjugated
β–catenin	E247	Abcam	AlexaFluor® 546*
Pan-cytokeratin (CK)	C11	Cell Signal	AlexaFluor® 555*
Epithelial cell adhesion molecule (EpCAM)	VU1D9	Cell Signal	AlexaFluor® 555*
Vimentin	V9	SCBT	AlexaFluor® 488/647
CD45	F10-89-4	AbD Serotec	AlexaFluor® 488/647

* refers to custom conjugated antibodies

Standard antibody incubation protocols were followed. After cells were permeabilized and blocked with serum, antibodies were added in a sequential manner; unconjugated antibodies first, secondary antibodies next, and finally, directly conjugated antibodies. Coverslips were mounted onto the slides with DAPI-containing mounting media. Images covering the whole cell spot was acquired on Nikon Eclipse 90*i* microscope, and image analysis for rare cell detection was performed using Definiens® software.

Results

Longitudinal monitoring of CTCs in a sarcoma patient during treatment

Fig. 1A

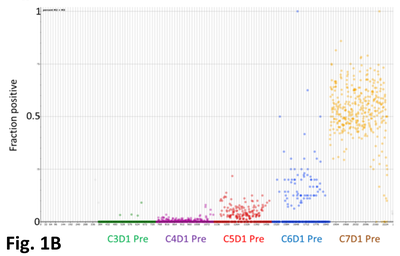


Fig. 1B

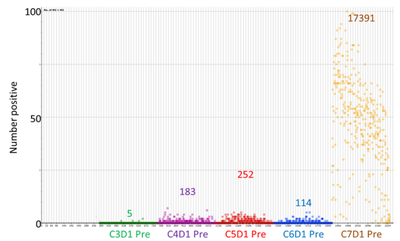


Fig. 1C

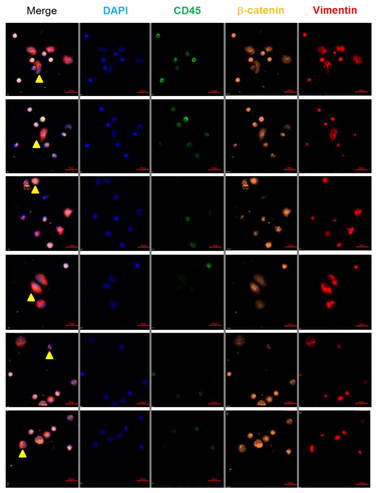
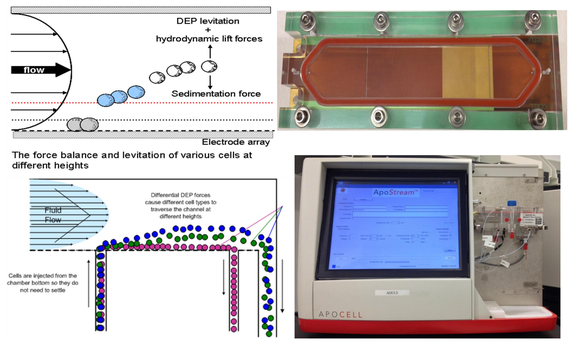


Fig. 1A. Time course measurement of the fraction of CD45(-) β–catenin(+) vimentin(+) cells identified per image field (x-axis). Each circle in the plot represents one image field.

Fig. 1B. Time course measurement of the number of CD45(-) β-catenin(+) vimentin(+) cells identified per image field (x-axis). Each circle in the plot represents one image field. Numbers noted in the graph represent the total number of cells identified from each cell spot, comprised of multiple image fields.

Fig. 1C. Image gallery of circulating non-hematopoietic cells with EMT phenotype isolated from a sarcoma patient using ApoStream® technology. Cells were stained for CD45 (green), β-catenin (orange), and vimentin (red). Yellow arrowheads point to nucleated CD45(-) β-catenin(+) vimentin(+) cells.

ApoStream® DEP-FFF



Results

CTC classification and enumeration in 4 carcinoma patients

Fig. 2A

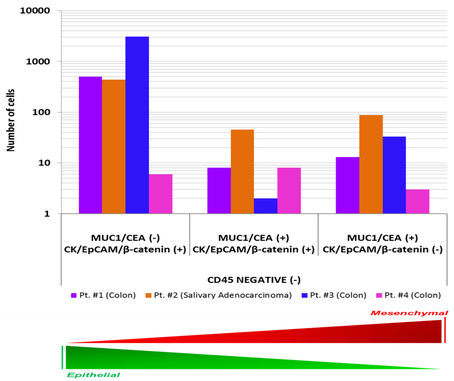


Fig. 2B

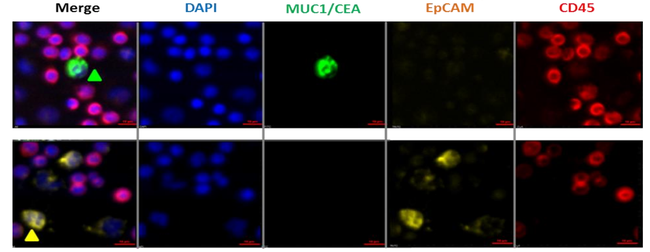


Fig. 2A. Enumeration and phenotypic characterization of CTCs from carcinoma patients. Y-axis denotes the total number of cells isolated (in log scale) with a specific phenotype (x-axis) from each patient specimen.

Fig. 2B. Image gallery of circulating non-hematopoietic cells with epithelial phenotype isolated from a carcinoma patient using ApoStream® technology. Cells were stained for MUC1/CEA (green), β-catenin/CK/EpCAM (yellow), and CD45 (red). Green arrowhead point to nucleated CD45(-) MUC1/CEA(+) cells. Yellow arrowhead point to nucleated CD45(-) CK/EpCAM/β-catenin (+) cells.

Summary and Conclusions

- We have previously reported on the use of a novel antibody-independent technology, ApoStream®, to isolate CTCs from alveolar soft part sarcoma patients [3].
- Here, we report preliminary results on the initial clinical readiness testing using specimens from patients with advanced sarcoma and carcinomas using this technology.
- A multiplex phenotyping assay (CD45, β-catenin, and vimentin) was developed to characterize circulating tumor cells isolated from patients with advanced sarcomas.
- Carcinoma CTCs were identified using an assay panel consisting of tumor-specific markers (MUC1 and CEA) and EMT markers (CK, EpCAM, and β-catenin).
- Definiens® software was used to develop an interim, user-defined analysis algorithm for rare cell detection, classification, and enumeration.
- Our current efforts are focused on evaluating the utility of ApoStream®-isolated CTCs for assessing the pharmacodynamic effects of anticancer agents on DNA damage response in patients with refractory solid tumors.

References

1. Park SR, Speranza G, Piekarz R, Wright JJ, Kinders RJ, et al. A multi-histology trial of fostamatinib in patients with advanced colorectal, non-small cell lung, head and neck, thyroid, and renal cell carcinomas, and pheochromocytomas. Cancer Chemotherapy and Pharmacology, April 2013, Volume 71, Issue 4, pp 981-990 .
2. http://us.gbo.com/bioscience/documents/flyers/217%20leucosep_update.pdf
3. Balasubramanian P, Wang L, Kummar S, Hollingshead M, et al. Fluorescent in situ hybridization confirmation of circulating alveolar soft part sarcoma cells (CTCs) isolated from peripheral blood specimens using ApoStream™ instrumentation. [abstract]. In: Proceedings of the AACR-NCI-EORTC International Conference: Molecular Targets and Cancer Therapeutics; 2013 Oct 19-23; Boston, MA. Philadelphia (PA): AACR; Mol Cancer Ther 2013;12(11 Suppl):Abstract nr C197.

Acknowledgments

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