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Isolation and Characterization of 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 manuscript 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. PRMC fractions were isolated following the manufacturer’s recommended protocol for CPT tubes or by Leucotrap® separation [2]. PRMC 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 Mammalert® slides, fixed, and stored at 4°C until further processing. All patients had written informed consent and were enrolled on NCIC Institutional Review Board (IRB)-approved protocols.

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. Coveralls were mounted onto the slides with DAPI-containing mounting media. Images covering the whole cell spot was captured on Nikon Eclipse 80i microscope, and image analysis for rare cell detection was performed using Deform® software.

Results

Longitudinal monitoring of CTCs in a sarcoma patient during treatment

CTC classification and enumeration in 4 carcinoma patients

Fig. 1A

Fig. 1B

Fig. 1C

Fig. 1D

Fig. 2A

Fig. 2B

Fig. 2C

Fig. 2D

Fig. 3A

Fig. 3B

Fig. 3C

Fig. 3D

Apostream® DEP-FFF

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 carcinomas.
- Carcinoma CTCs were identified using an assay panel consisting of tumor-specific markers (MUC1 and CEA) and EMT markers (CK, EpCAM, and β-catenin).

Deform® 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


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