AUTHORS and AFFILIATIONS:
Jinling Zhang, a Jose I. Varillas, b Kangfu Chen, a Chen Liu, c Thomas J. George Jr. d,e and Z. Hugh Fan a,b,e*

a Interdisciplinary Microsystems Group, Department of Mechanical and Aerospace Engineering, University of Florida, PO Box 116250, Gainesville, Florida 32611, USA. E-mail: hfan@ufl.edu; Fax: +1 352 392 7303; Tel: +1 352 846 3021;
b J. Crayton Pruitt Family Department of Biomedical Engineering, UF
c Department of Pathology, Immunology and Laboratory Medicine, UF
d Department of Medicine, UF, P.O. Box 100278, Gainesville, FL 32611, USA. E-mail: thom.george@medicine.ufl.edu
e Department of Chemistry, UF

TITLE
Detection and analysis of circulating tumor cells and cancer stem-like cells from patients with pancreatic ductal adenocarcinoma using microfluidic devices

HYPOTHESIS:
The treatment of pancreatic ductal adenocarcinoma (PDAC) requires multimodal therapeutic approaches and need for monitoring tumor plasticity. Liquid biopsy biomarkers, including circulating tumor cells (CTCs) and cancer stem-like cells (CSCs), hold promise for evaluating treatment response in real-time and guiding therapeutic modifications. Antibody-functionalyzed microfluidic devices showed advances in a malignancy where CTCs are not routinely detected by current commercial CTC assays. Using the EpCAM or the stem cell marker CD133 antibody immobilized microfluidic chip, we can detect CTCs and CSCs from patients' blood sample to monitor treatment responses.

BACKGROUND/AIMS:
The spread of cancer cells from the primary tumor site to distant tissues is an early event in the metastatic process. CTCs have been defined as cancer cells of solid tumor origin found in the peripheral blood and are considered to be the roots of metastasis. A number of studies have linked CTCs to tumor progression in a variety of solid tumors. The presence and frequency of CTCs in the blood of patients with epithelial carcinomas provides valuable insights associated with disease stage and treatment evaluations. These rare cells correlate with distant metastases, prognosis and treatment effect. Such correlations are lacking for PDAC owing to exceptional cell rarity.

Many current technologies rely on epithelial phenotype-specific markers to capture and identify CTCs. However, those markers limit CTC detection rates and underestimate CTC heterogeneity because they may fail to identify cancer cells that have partially or completely lost their epithelial phenotype due to epithelial-to-mesenchymal transition (EMT). There is also considerable evidence to support the presence of CSCs, an ultra-rare population of cells relative to the total tumor bulk. CD133+ CSCs have been identified in many types of cancer. The expression of the CD133 protein has been demonstrated to increase the tumorigenic potential and treatment resistance of tumor cells in these cancers. The ability to detect CSCs as a component of CTCs may serve as an important prognostic and treatment monitoring tool.

METHODS:
From 33 patients with metastatic PDAC undergoing treatment, we collected 74 blood samples at different time points for CTC and CSC isolation using EpCAM or/and the stem cell marker CD133 antibody functionalized microfluidic platform. The number of CTC and CSC were analyzed for each patient during the treatment. The CTC subtypes (e.g., CD133 positive or CD133 negative CTCs) for pancreatic cancer patients' peripheral blood were also analyzed. The knowledge and information gained from the clinical blood samples helped us evaluate the clinical response of patients to the treatment.

RESULTS & CONCLUSIONS:
The majority (93.2%) of patient blood samples were positive for CTCs and 81.2% of patient blood samples were positive for CSCs, using a healthy baseline value as threshold (0.4 CTCs or CSCs/mL). CD133 positive CTC were also analyzed. Among 11 samples, 27.3% of captured CTCs were CD133+ CTCs. In several cases, CSCs exhibited cytokeratin expression that was not detected using EpCAM antibody functioned devices.

This method enabled the reliable isolation of CTCs and CSCs from PDAC patient samples, as well as the CD133+ CTC subtypes. Interestingly, complementary assessment of both CTCs and CSCs appears advantageous to assess the progressing of tumor. We have found in several patients, the CTC and CSC number in the peripheral blood decreased with effective anticancer treatment, and the numbers increased as cancer relapses. This research has important implications for the application and interpretation of approved methods to use CTCs and CSCs as prognostic markers for real-time monitoring of cancer patients.