ApoStream™ Isolated Circulating Tumor Cells from Primary Breast Cancer Patients Reveals Heterogeneous Phenotypes Related to Epithelial-Mesenchymal Transition and Stem Cell Markers

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Abstract

Background: Detection of circulating tumor cells (CTCs) is an indicator of poor prognosis in patients with metastatic breast cancer and not in primary breast cancer (PBC). The classical phenotypic definition of CTC is a mammoth (CD45−) cell that co-expresses (CK) positive and CSC markers. Recent reports have shown that epithelial cell adhesion molecule (EpCAM) based capture methods detect only a fraction of CTCs present in primary breast cancer patients. The current study aimed to assess the CTC population in PBC using a novel method and compare the CTC phenotype to metastatic breast cancer patients.

Study Design

Blood Collection

CTC isolation

ApoStream™

Immobilize cells on slides

CTC/CSC staining

CK+CD45-DAP+ Enumeration

iCytes Laser Scanning Cytometer

Biomarker Expression in CTCs

Patient ID % EpCAM+/Vimentin+ among CK+/CD45- population

CSC Subpopulation of CTCs

CD24+/CD44high

Patient ID % CD24+/CD44+ cells among CK+/CD45- population

Summary

• CTCs (CK+/CD45-DAP+) cells were detected in 71% (10/14) primary breast cancer patients prior to receiving preoperative therapy. 

• EMT and stem cell markers range of expression and frequency of detection in PBC patients:
  - EpCAM+ range: 2-9.4% in 78% (7/10) patients
  - β-Catenin range: 6.37% in 21% (3/14) patients
  - EpCAM-Vimentin was 3% in 14% (2/14) patients
  - CD24+/CD44+ range: 8.75% in 71% (8/14) patients

• In this ongoing clinical trial, we will test the hypothesis that low EMT-CTC and CSCs in baseline blood samples is correlated with a higher pCR rate compared to PBC patients with high EMT-CTC and CSC counts.

References


Current prototype design shipped to National Cancer Institute & Massey Cancer Center, VA, in Dec 2012

Figure 1. CTCs (defined as CK+/CD45− DAPI− cells) were enumerated in samples collected from 14 PBC patients. CTCs were enumerated from 7 samples, and the number of CTCs ranged from 0 to 185 with the average count per patient ranging from 5 to 91.

Figure 2. The CTC population was analyzed for expression of EpCAM (Vimentin) and CSC (CD44+/CD44+) markers. Expression levels of each biomarker was calculated as (Rho bphy (Fls) expressing marker positive in %/CTC) x 1000. Representative images shown below.

Figure 4. CTCs were analyzed for the expression of CD24+/CD44+. Eight of 14 (57%) patients had CD24+/CD44+ CTCs, ranging from 6.75.

Figure 3. The CTC population was analyzed for EpCAM-Vimentin expression, which has been shown to signify the EMT state. Two of 14 (14%) patients were shown to express EpCAM-Vimentin cells, ranging from 2.3%.