Identification of circulating tumor cells captured by the FDA cleared Parsortix® PCI System from the peripheral blood of metastatic breast cancer patients using immunofluorescence and cytopathological evaluations

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Introduction

Circulating tumor cells (CTCs) captured by the blood of cancer patients may serve as a non-invasive source of tumor material to investigate tumor characteristics in real-time. The Parsortix® PCI System, the first FDA-cleared medical device for the capture and harvest of CTCs from the peripheral blood of metastatic breast cancer (MBC) patients, is under prospective randomized downstream analyses, enabling the epitope independent capture of CTCs with diverse phenotypes based on cell size and deformability.

The Parsortix® PCI instrument is designed for use with a Parsortix® GEN3 Cell Separation Cassette (Figure 1). This study aimed to determine the proportion of MBC patients and self-declared female healthy volunteers (HV) that had one or more CTCs identified in the population of cells harvested from their peripheral blood by the Parsortix® PCI System using an immunofluorescence (IF) based assay for detection of epithelial CTCs followed by CTC identification using Wright-Giemsa staining and cytomorphological review.

Materials and Methods

75 HVs and 77 MBCs patients included

Blood processed on Parsortix® PCI Systems within 8 hours after collection

Harvested cells deposited onto a cytology slide using Cytospin method

Staining, imaging and analysis using ANGLE’s Epithelial IF assay: Pan-CK (FITC) + EpCAM (C5) + Blood lineage markers (C5) + Nuclear dye (DAPI)

Re-staining using Wright-Giemsa followed by brightfield imaging and cytology analysis

Peripheral blood from 75 HVs and 77 patients with MBC was prospectively collected into K2EDTA tubes at the University of Rochester Medical Center for this study. Assay processes and analyses are detailed in Figure 2. All laboratory testing and analysis was performed by operators blinded to the clinical status of each subject. First, CTCs were defined using IF staining as nucleated cells (DAPI+) that were positive for CK and/or EpCAM and negative for the blood lineage markers. Slides were then restained using Wright-Giemsa staining and morphological characteristics of CTCs were used by a qualified pathologist to define and identify CTCs.

Results

On the evaluable IF-stained slides (75 MBC and 71 HVs - Figure 3A-B):

- CTCs were identified in 45.3% of the MBC patients (range = 0 – 125, mean = 7) and in 5.6% of the HVs (range = 0 – 8, mean = 0).

- In the 38 MBC patients with one or more CTCs observed, 76.6% had only CK+, EpCAM-cells while the remaining 23.4% had at least 1 CK+, EpCAM+ cell. No EpCAM-, CK- CTCs were identified in either the MBC patients or HVs.

On the evaluable Wright-Giemsa stained slides (70 MBC and 68 HVs - Figure 3C), cells classified as CTCs by the pathologist were identified in 42.9% of the MBC patients (range = 0 – 41, mean = 4) and in 4.4% of the HVs (range = 0 – 14, mean = 0).

Conclusions

- ANGLE’s Parsortix® PCI System can capture and harvest CTCs from a significantly larger proportion of MBC patients compared to HVs and the harvested cells can be successfully evaluated using both IF staining and Wright-Giemsa cytomorphological assessment.

- High proportion of CTCs did not express EpCAM, further highlighting limitations of using EpCAM-based approaches to capture CTCs.

- It was demonstrated at ANGLE that the use of cytospin causes up to 70% cell loss. Therefore, these results substantially understate the number of CTCs harvested by Parsortix® PCI instrument. ANGLE is currently developing alternative processing methods with limited cell loss for downstream imaging assays.

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