**Detection of circulating tumor cells and HER2 gene amplification status in breast cancer using a novel microfluidic platform (Cell Enrichment and Extraction Technology, CEE™)**

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**INTRODUCTION:** The rarity of circulating tumor cells (CTCs) in blood poses challenges in their detection. The majority of techniques utilized for the detection of CTCs are limited in their ability to allow detailed phenotypic and genotypic evaluation of the CTCs. In this pilot study, we report the utility of a novel microfluidic platform utilizing cell enrichment and extraction technology (CEE™, Biocept, Inc., San Diego) for detection of CTCs in breast cancer and subsequent evaluation of HER2 gene amplification status of the CTCs by fluorescent in situ hybridization (FISH).

**METHODS:** Peripheral blood (10 mL) was collected from patients with operable breast cancer in a prospective institutional review board approved protocol. Mononuclear cells were recovered using a Percoll density gradient method, incubated with a mixture of 10 primary capture antibodies (cAbs) and then introduced into microchannels (CEE channels). The cells in the channels were stained using fluorescent permeabilizer (DPX) or in addition to a fluorescent probe to detect cAb on the cells. Cells were stained for cAb as a negative control. The enumeration and localization of cAb-CK+, cAb-HER2, and cAb-CD45 as well as cAb-CD45- cells were performed by microscopic examination of the microchannels. Finally, the microchannels were processed for FISH analysis using three direct labeled probes specific for centromere 8 (spectra green), 17 (spectra green) and HER2 (spectra orange). The ratio of HER2:CEP 17 ≥ 2:1 in any cAb-negative cell was regarded as positive for HER2 gene amplification.

**RESULTS:** Peripheral blood from 25 patients with T1NO(1), T1N1(2), T2N1(3), T2N2(2), T2N3(1), T3N1(2), T4N1(2), T4N2(1) showed cAb-CK+, cAb-CD45- CTCs in 75% (19/19), 66.6% (2/3), 33.3% (1/3) of the first three, respectively, and in all the patients in the remaining groups. Among the different genomic groups of the primary tumor, cAb-CK+, cAb-HER2, cAb-CD45- were detected in 79% (19/19) Luminal A, 60% (12/20) Luminal B, 50% (2/2) HER2 and 100% (1/1) of triple negative tumors. Overall 5/5 of the primary tumors were HER2 amplified. While cAb-CK+ cells were isolated in 3/5 of these patients, HER2 amplification was detected in a cAb-CK+, cAb-HER2, cAb-CD45- in only one patient with a Luminal B tumor. In addition, amplified cAb-HER2 CTCs were also detected in a patient with primary tumor negative for HER2. The two patients with HER2+ CTCs were therefore cAb-CK-, cAb-CD45-.

**CONCLUSION:**
1. The cell enrichment and extraction microfluidic technology (CEE™) provides a sensitive platform for enhanced detection and characterization of antibody-cytokeratin positive and negative CTCs.
2. This platform allows evaluation of HER2 gene amplification status by FISH in intact CTCs within the microchannels.
3. The utility of this platform for phenotypic and genotypic characterization of CTCs in breast cancer needs to be tested in larger clinical trials.

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