Detection of HER2 gene amplification in circulating tumor cells (CTCs) and disseminated tumor cells (DTCs) in bone marrow by fluorescence in situ hybridization using OncoCEETM

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Introduction

The occurrence of circulating tumor cells (CTCs) in blood and disseminated tumor cells (DTCs) in bone marrow (BM) in patients with early and advanced breast cancer needs to be evaluated prospectively in larger clinical trials. The complex process of metastasis. The detection of these cells is challenging due to their extremely rare occurrence in peripheral blood. The OncoCEETM microfluidic platform has been developed and shown to efficiently capture CTCs based on standard cytokeratin(CK)-CEP17-DAPH staining criteria. The system enables sequential recovery of CTCs and DTCs, stained with fluorescent anti-cytokeratin (CK) and anti-CD45 antibodies and subsequently processed for multi-color FISH using the FDA-approved PathVysion HER2 DNA Probe Kit consisting of two direct-labeled probes (Abbott Molecular, Abbott Park, IL) specific to HER2 and CEP17. Sections of the primary tumor were fixed in buffered formalin, immediately processed for fluorescence in situ hybridization (FISH) analysis of the recovered cells. We report here the utility of the OncoCEETM device for detecting HER2 gene amplification by FISH in stashed CTCs and DTCs in patients with operable breast cancer.

Materials & Methods

Peripheral blood and/or BM from 107 patients (88 BM; 99 blood; 79 matched blood and BM) were collected from patients with operable breast cancer in a prospective institution review board-approved protocol. Peripheral blood was sampled using 9 mL acid-citrate-dextrose solution (ACD solution A; Vacutainer; BD, Franklin Lakes, NJ) and centrifuged three times at 400G for 10 minutes following washings with PBS/Casein/EDTA. Peripheral blood and/or BM were collected from patients with HER2-negative primary tumors who have HER2-positive CTCs and/or DTCs. The majority of techniques utilized for detection of residual disease are limited in their ability to allow detailed phenotypic and genotypic evaluation of the cells. We report here the utility of a microfluidic platform (OncoCEETM; Biocept, Inc., San Diego, CA) for detection of HER2 gene amplification in CTCs and DTCs in patients with non- metastatic breast cancer.

RESULTS:

Peripheral blood and/or BM from 107 patients (88 BM; 99 blood; 79 matched blood and BM) were collected from patients with operable breast cancer in a prospective institution review board-approved protocol. Peripheral blood was sampled using 9 mL acid-citrate-dextrose solution (ACD solution A; Vacutainer; BD, Franklin Lakes, NJ) and centrifuged three times at 400G for 10 minutes following washings with PBS/Casein/EDTA. Peripheral blood and/or BM were collected from patients with HER2-negative primary tumors who have HER2-positive CTCs and/or DTCs. The majority of techniques utilized for detection of residual disease are limited in their ability to allow detailed phenotypic and genotypic evaluation of the cells. We report here the utility of a microfluidic platform (OncoCEETM; Biocept, Inc., San Diego, CA) for detection of HER2 gene amplification in CTCs and DTCs in patients with non-metastatic breast cancer.

CONCLUSION:

1. OncoCEETM provides a sensitive platform for evaluation of HER2 gene amplification of CTCs and DTCs.

2. HER2-positive CTCs and/or DTCs occurred in 25% of operable patients with HER2-negative primary tumors when a HER2 ratio cut-off of ≥ 2.0 was applied. The discordance rate in detecting HER2-positive CTCs and/or DTCs in operable patients with HER2-negative breast cancer needs to be evaluated prospectively in larger clinical trials.

3. The clinical significance of evaluating the status of HER2 gene amplification in CTCs and DTCs with breast cancer needs to be evaluated prospectively in larger clinical trials to assess its efficacy in treating patients classified as HER2-positive by CTC/DTC analysis.

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