
# FISH-based determination of HER2 status in circulating tumor cells isolated with the microfluidic CEE™ platform

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## ABSTRACT

**Purpose:** Determination of HER2 status by immunohistochemistry (IHC) and fluorescent in-situ hybridization (FISH) in patients with breast cancer is considered standard practice for selection of treatment options. Though patients presenting with recurrent and/or metastatic disease are often re-evaluated for HER2, biopsy is not often feasible. Thus, circulating tumor cells (CTCs) are an attractive alternative source of tumor tissue for determining HER2 status to enable a more effective course of treatment.

**Experimental Design:** Twenty to thirty ml of peripheral blood was collected prospectively from 54 patients diagnosed with late stage metastatic/recurrent breast cancer. CTCs were isolated using the microfluidic CEE™ platform. CTC capture was achieved using a cocktail of capture antibodies, followed by detection with an expanded anti-cytokeratin (CK) cocktail mixture and anti-CD45. HER2 amplification was subsequently assessed by FISH on captured CK+/CD45- and CK-/CD45- cells.

**Results:** CK+/CD45- cells were detected in 43 of 54 cases (80%). Among the 43 cases in which CK+ cells were detected, high concordance (93%) in HER2 status between primary tumor (by IHC and FISH) and CTCs (by FISH) was observed. An overall sensitivity of 95% and a specificity of 92% were obtained using the OncoCEE-BR™ assay.

**Conclusions:** Recovery of CTCs from peripheral blood using the CEE™ platform is shown to be efficient and suitable for FISH-based testing. In addition, HER2 FISH on recovered CTCs is proven to be sensitive and accurate.

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## INTRODUCTION

Breast cancer (BC) is a heterogeneous disease, encompassing a number of distinct biological entities that are associated with specific morphological and immunohistochemical features and clinical behavior.

The presence of circulating tumor cells (CTCs) in peripheral blood of breast cancer patients has long been associated with metastasis and poor survival[1-3].

While technical advances have made it possible for the detection of CTCs in whole blood, current techniques are limited in their capture efficiency and the ability to allow detailed phenotypic and genotypic evaluation of the CTCs[4].

To utilize CTCs clinically in a more comprehensive and sensitive manner there remains a need for a methodology that can efficiently capture, enrich, and subsequently improve the detection rates of CTCs in blood.

In this prospective study, we report the utility of a microfluidic platform that utilizes CEE™ (cell enrichment and extraction technology; Biocept Inc., San Diego, CA) for capture, enrichment, and subsequent molecular evaluation of HER2 amplification status in CTCs by fluorescent in situ hybridization (FISH).

## METHODS AND MATERIALS

***Laboratory Information and Patients*** Patients with advanced stage breast cancer were enrolled from June 2010 to November 2010. Peripheral blood was collected under appropriate third party institution review board approved protocols (Conversant Laboratories & BioOptions).

***Cell Separation*** Leucosep tubes (Greiner bio-one, Monroe, NC) utilizing a Percoll density gradient method were used to recover the peripheral blood mononuclear cell fraction (PBMC). Fc blocker (100ug/mL) and a capture antibody cocktail (1µg/mL of each antibody in the cocktail) were added for 30 minutes at room temperature to the recovered PBMC fraction. Following a wash and centrifugation, biotinylated secondary antibody was added for 30 minutes at room temperature. Following washing the final pellets were subsequently applied to the CEE™ microchannels.

***CTC Enrichment and Detection*** CEE™ microchannels are manufactured at Biocept, Inc. (San Diego, CA). The cell fraction is run through the microchannel and the captured cells stained with a mixture of anti-cytokeratin antibodies labeled with AlexaFluor-488. Cells were simultaneously stained with anti-CD45 labeled with AlexaFluor-594. The microchannels undergo microscopic analysis for enumeration of CK+/CD45-/DAPI+ (criteria for CTC identification), CK-/CD45+/DAPI+ (criteria for background white blood cells) and CK-/CD45-/DAPI+ (possible CTCs that lack CK) cells.

***Fluorescent in situ hybridization*** Following CTC enumeration, the CEE™ microchannels were processed for multi-color FISH using the FDA-approved PathVysion HER2 DNA Probe Kit (centromere 17 specific probe, CEP 17-Spectrum Green; and locus specific HER2 probe, Spectrum Orange) and a centromere specific probe to chromosome 8 (CEP 8, Spectrum Aqua, Abbott Molecular, Abbott Park, IL) for use as an internal control for ploidy status. The ratio of HER2:CEP 17 was calculated and a ratio >2.2 in any CD45 negative CK+ or CK- cell was regarded as positive for HER2 gene amplification.

## RESULTS

A total of 54 peripheral blood samples from patients with defined HER2 tissue status were collected and processed for CTC enumeration and HER2 FISH characterization. Patient characteristics are shown in **Table 1**.

Following enumeration, 43 patients were found to have ≥1 CTC identified based on a staining pattern of CK+/CD45- cells (prevalence of 80% based on CK+ staining).

**Table 1. Patient Characteristics**

| Characteristic, n (%) | All Patients n = 54 | Patients with ≥ 1 CTC (n = 43) |
|---|---|---|
| **Median Age, Years (Range)** | 64 (29-86) | |
| **CTCs based on CK+** | | |
| 0 | 11 (20) | |
| 1-4 | 15 (28) | |
| 5-9 | 9 (17) | |
| ≥ 10 | 19 (35) | |
| **HER2+** | 24 (44) | 20 (47) |
| **HER2-** | 30 (56) | 23 (53) |
| **ER+/PgR+** | 31 (57) | 26 (60) |
| **ER+/PgR-** | 13 (24) | 10 (23) |
| **ER-/PgR-** | 10 (19) | 8 (19) |
| **Ethnicity Breakdown** | | |
| White (non-Hispanic) | 41 (76) | |
| African American | 4 (7) | |
| Hispanic | 2 (4) | |
| Asian Pacific Islander | 2 (4) | |
| Other | 4 (7) | |

Abbreviations: CK = cytokeratin; CTC = circulating tumor cell; ER = estrogen receptor; n = node; PgR = progesterone receptor

**Table 2. Concordance of HER2 Status Between CTCs isolated using the OncoCEE-BR™ and Primary Tumor**

| Primary Tumor | CTCs, n (%) | | |
|---|---|---|---|
| | HER2- | HER2+ | Total, n |
| **HER2-** | 22 (51) | 2 (5) | 24 |
| **HER2+** | 1 (2) | 18 (42) | 19 |
| **Total** | 23 (53) | 20 (47) | 43 |

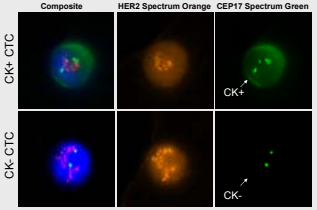
Abbreviation: CTC = circulating tumor cell, n = node

## RESULTS

Among the 43 CK+ informative cases, an overall concordance of 93% between CTCs and the primary tumor was observed with regards to HER2 status. Concordance was observed in 18 of 19 cases with positive HER2 status (positive predictive value of 90%) and 22 of 24 cases with negative HER2 status (negative predictive value of 96%). There was no difference between the numbers of CK+ CTCs detected in the 19 patients with HER2+ status compared to the 24 HER2- cases (median 5 and 9 CTCs, respectively, p = 0.37). Discordance was observed in three cases as shown in **Table 2**.

An overall test sensitivity of 95% and specificity of 92% was observed. A substantial agreement approaching the range of perfect agreement (0.81-1.00) was found using the Cohen's k statistic (k= 0.75) for concordance between HER2 status in the primary tumor and CTCs.

**HER2 Amplification in CTCs isolated using CEE™**




An important observation was made during the course of this study, which is that HER2 amplified cells enriched using the CEE™ platform were both CK+ and CK- (**Figure 1**).

These results suggest that FISH detection of abnormal chromosomal content or structure in CTCs is feasible following enrichment and capture using CEE™, and can be used to confirm a CK-/CD45- cell as a tumor cell.

## DISCUSSION

The CEE™ platform utilizes an antibody cocktail to enable capture of a more heterogeneous CTC population based on the identification of HER2 gene amplification by FISH in both CK+/CD45- and CK-/CD45- cells.

These results strongly indicate that CK-/CD45- cells are in fact CTCs that have presumably down-regulated CK expression below visible detection levels. Thus, studies that are limited to the use of EpCAM and CK for capture/enrichment and detection of CTCs, respectively, may not observe significant concordance of tumor biomarkers such as HER2 between CTCs and primary tumor.

Though some discordance between tumor and CTCs is perhaps expected given tumor heterogeneity, biopsy size, and robustness of the technical assay (especially for IHC), a blood-based CTC assay may offer more reliable testing given the advantages of simple repeat testing the use of larger blood volumes when needed to ensure informative results.

## CONCLUSIONS

The CEE™ technology provides a sensitive platform for enhanced capture, detection and characterization of both CK+ and CK- CTCs.

This platform allows for evaluation of HER2 gene amplification status by FISH in intact CTCs within the microchannels at sensitivity and accuracy levels suitable for standardized CLIA-laboratory testing.

## REFERENCES

1. Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 2004;351(8): 781-91.
2. Cristofanilli M, Hayes DF, Budd GT, et al. Circulating tumor cells: a novel prognostic factor for newly diagnosed metastatic breast cancer. J Clin Oncol 2005;23(7): 1420-30.
3. Fehm T, Sagalowsky A, Clifford E, et al. Cytogenetic evidence that circulating epithelial cells in patients with carcinoma are malignant. Clin Cancer Res 2002;8(7): 2073-84.
4. Paterlini-Brechot P, Benali NL. Circulating tumor cells (CTC) detection: clinical impact and future directions. Cancer Lett 2007;253(2): 180-204.