INTRODUCTION

Determination of HER2 status (IHC) and enable capture of a more heterogeneous CTC patients with defined HER2 tissue status were 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 98%). 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 (0.91-1.00) was found using the Cohen’s k statistic (k= 0.75) for concordance between HER2 status in the primary tumor and CTCs.

METHODS AND MATERIALS

Laboratory Information and Patients Patients with advanced stage breast cancer were recruited from the Breast Cancer Program at Biocept from September 2010 to December 2010. Patients were enrolled under institutional review committee approved protocols (Conversant Laboratories & BioOptions).

Cell Separation Leukocyte lysis (Genta), lone, NC) allowing a Percoll density gradient method were used to recover the peripheral blood mononuclear cell fraction (PBMC). Fc blocker (1µg/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, bismaleimide 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 with a clinical sample. The capture cells are tagged with a mixture of anti-cytokeratin antibodies labeled with AlexaFluor 488. Cells were simultaneously stained with anti-CK labeled with AlexaFluor 568. The microchannels undergo microscopic analysis for enumeration of CK+/CD45- CTCs (positive for background white blood cells) and CK+/CD45+ (positive 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 Spectral Profile kit for HER2 analysis (Abbott Oncology, Abbott Park, IL). This 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.

RESULTS

Experimental Design: Twenty to thirty ml of peripheral blood was collected prospectively from 54 patients diagnosed with late stage metastatic 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 CD45+ cells.

Results: CK+/CD45- cells were detected in 18 of 54 cases (33%). 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 specificity of 92% was observed using the OncorCE TEST.

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

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.

Among the 42 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 98%). 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 (0.91-1.00) was found using the Cohen’s k statistic (k= 0.75) for concordance between HER2 status in the primary tumor and CTCs.

CONCLUSIONS

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


Julie Ann Mayer, PhD; Tony J. Pircher, PhD; Stephen D. Mikolajczyk, BS; Philip D. Cotter, PhD, FACMG; and Farideh Bischoff, PhD
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FISH-based determination of HER2 status in circulating tumor cells isolated with the microfluidic CEE® platform

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