Circulating Tumor Cell (CTC) Biomarker Evaluation from Patients with Metastatic Breast Cancer (MBC) Utilizing the Target Selector™ Platform

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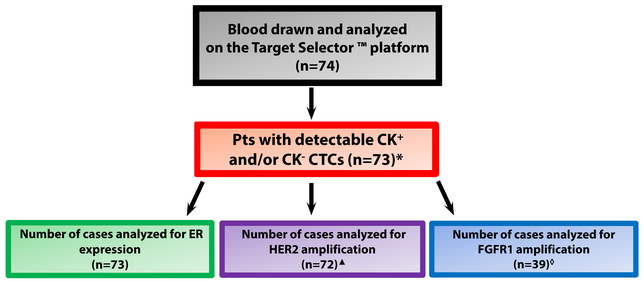
BACKGROUND

- Circulating blood biomarkers represent the promise of non-invasive, real-time surrogates for tumor tissue-based biomarkers. They also afford the opportunity to monitor the evolution of tumor cells and acquired resistance to treatment over time.
- Circulating tumor cells (CTCs) are cells that disseminate from tumors and recent advances in this arena have permitted the enrichment and detection of CTCs in peripheral blood.
- The Target Selector™ and the FDA-approved CellSearch® platforms are emerging tools for the detection and characterization of CTCs.
- In contrast to the FDA-approved platform, Biocept's Target Selector™ platform utilizes a proprietary antibody capture cocktail with a novel microfluidic system that enables enrichment, enumeration, and characterization of CTCs, including cytokeratin- positive (CK^+) and cytokeratin-negative (CK^-) CTCs, thus detecting a broader range of CTC phenotypes.
- Therefore, the objective of this pilot study is to detect expression of ER, and amplification of HER2 and FGFR1 on CTCs (CK^+ and CK^-) isolated from metastatic breast cancer (MBC) patients using the Target Selector™ platform.

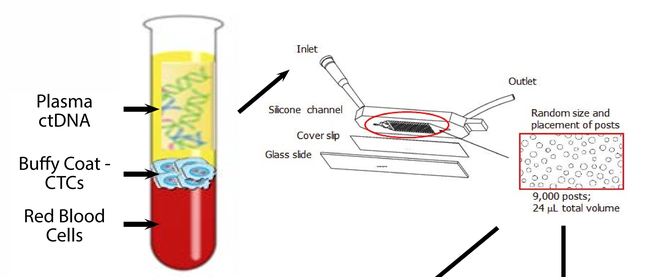
METHODS

- Seventy-four (74) ER^+ and $HER2^+$ MBC patients (pts) were consented for this study.
- Archival tumor tissue from the most recent biopsy (primary tumor or metastatic lesion) was used for standard biomarker analysis.
- Blood was drawn into Biocept's proprietary CEE-Sure™ blood collection tubes from consented patients for utilization in the Target Selector™ platform.
- Biomarker expression on captured CTCs was determined by immunofluorescence (IF) for ER and by fluorescence *in situ* hybridization (FISH) for HER2 and FGFR1. Concordance between these results and biomarker expression on archival tumor tissue was calculated.

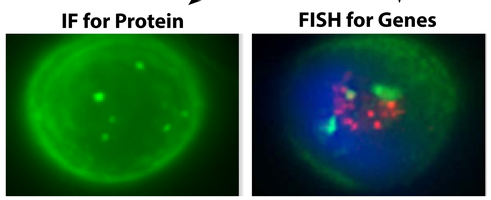


*CTCs were not detected in 1 pt
▲HER2 data not available for 1 pt
◊FGFR1 data not available for 34 pts

TARGET SELECTOR™ PLATFORM



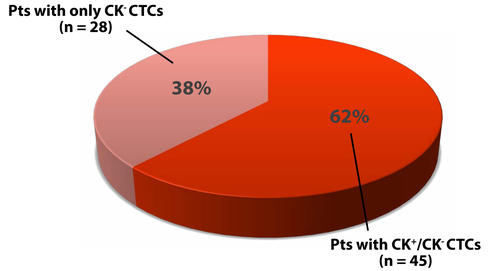
- CTCs are captured in transparent microfluidic chambers and can be veiwed *in situ* by fluorescent microscopy.
- CTCs can be analyzed via IF and FISH.



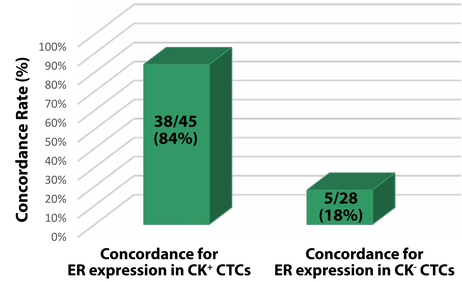
RESULTS

- Based on analysis from the most recent tumor biopsy, 93% (69/74) of the MBC pts had ER^+ breast tumors, 16% (12/73) had HER2 amplification and 20% (8/40) had FGFR1 amplification in their tumor.
- CTCs were detected in 73 out of 74 (99%) pt blood samples (range, 2-4471); 62% (45/73) had both CK^+ and CK^- CTCs, and 38% (28/73) had only CK^- CTCs. None had only CK^+ CTCs.
- Of those pts with CK^+ CTCs, concordance for ER expression (+ or -) between tissue and blood analyses was 84% (38/45). Concordance was much lower for pts with only CK^- CTCs (18%, 5/28).
- Concordance for HER2 amplification in pts with CK^+ CTCs was 93% (41/44) and 68% (19/28) in pts with only CK^- CTCs.
- FGFR1 amplification data was available for 39 pts. Concordance for FGFR1 amplification was 79% in pts with CK^+ CTCs (19/24) and 67% in pts with CK^- CTCs (10/15).

PREVALENCE OF CK^+ and CK^- CTCs

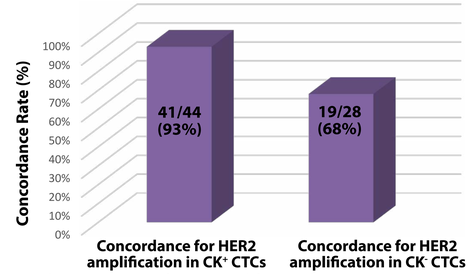


CONCORDANCE RATE OF ER EXPRESSION (IHC/IF)



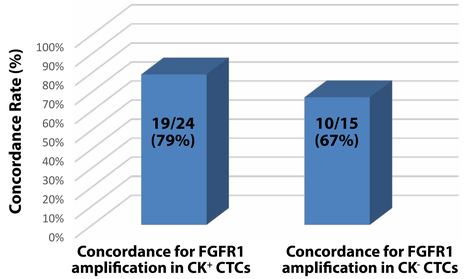
Concordance rates (%) of ER expression between CTCs in the peripheral blood of MBC pts and archival tumor tissue.

CONCORDANCE RATE OF HER2 AMPLIFICATION (FISH)



Concordance rates (%) of HER2 amplification between CTCs in the peripheral blood of MBC pts and archival tumor tissue.

CONCORDANCE RATE OF FGFR1 AMPLIFICATION (FISH)



Concordance rates (%) of FGFR1 amplification between CTCs in the peripheral blood of MBC pts and archival tumor tissue.

DISCUSSION

- CTCs were discovered in nearly all MBC pts that participated in this study. CK^+ CTCs were found in the majority of pts (62%).
- For this study, there was a latency period between blood draws and tissue collections for biomarker assessments. This latency period may have influenced concordance levels.
- Concordance of all biomarkers (ER, HER2, and FGFR1) was higher when cytokeratin-positive CTCs were present in the blood sample. This may represent a variety of factors including phenotypic variability in cytokeratin-negative cells undergoing epithelial to mesenchymal transformation (*i.e.*, downregulation of proteins).
- The difference in concordance between cytokeratin-positive and cytokeratin-negative CTCs was particularly pronounced using the cell surface IF assay for ER, and less marked using FISH-based assays for both HER2 and FGFR1. The significance of cytokeratin-negative cells as a potential prognostic indicator to assess ER, HER2, and FGFR1 biomarkers requires further evaluation.
- Blood-based testing for CTCs affords an opportunity to assess biomarker status in real time during treatment as well as in circumstances when tissue is not readily available or when tissue processing (*i.e.*, decalcification of bone) makes biomarker testing less reliable.

SUPPORT

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Disclosure: Lan Huynh, BS and Veena M. Singh, MD are employed by Biocept, Inc.