Using a Liquid Biopsy, Circulating Tumor Cell Based Approach for Determination of HER2 Amplification Status in Patient Samples

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Introduction

- Amplification of HER2 is an important therapeutic and prognostic biomarker.[1] Advances in HER2 targeted therapy have led to improvements in outcomes in patients with HER2+ (FISH+, IHC3+) and $HER2_{low}$ (FISH-, IHC 1+ or 2+) disease.[2]
- Standard of care approach for determination of HER2 status relies on tissue biopsy. However, HER2 signature can change overtime and is heterogeneous across disease sites.[3]
- A liquid biopsy-based approach is a non-invasive, quick, and potentially less biased way for determining HER2 status.[4]
- We provide results from a circulating tumor cell (CTC) based HER2 amplification analysis of patients with biopsy-proven HER+, $HER2_{low}$, or $HER2_{null}$ (FISH-, IHC 0) breast cancer.

Objectives

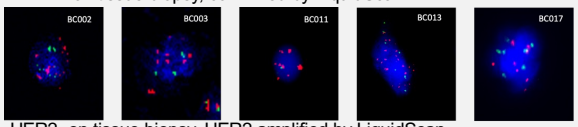
- Determine the feasibility of detecting HER2 amplified CTCs in whole blood samples of patients with biopsy proven HER2+ localized and metastatic breast cancer (MBC).
- Compare HER2 amplification status using CTCs to tissue biopsy results in patients with HER2+, $HER2_{low}$, and $HER2_{null}$ breast cancer
- Assess prognostic implications of the presence vs absence of HER2 amplified CTCs in metastatic $HER2_{low}$ breast cancer.

Methods

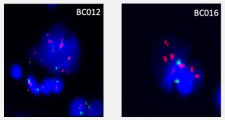
Sample Collection: Whole blood from 27 consenting patients with biopsy proven stage II-IV breast cancer was collected at diagnosis for localized disease, and at diagnosis or progression for metastatic disease.
CTC Isolation: Whole blood is processed by the LiquidScan device via microfluidic chips with channels coated with antibody to human epithelial cell adhesion molecule. Non-immobilized cells are washed away. Remaining cells are released from the chip using a restriction enzyme then deposited onto slides.
CTC and FISH Analysis: Cells were stained for pan-cytokeratin, CD45, and with the nuclear dye Hoechst 33342 to identify CTCs (cytokeratin+, CD45-). Probes for HER2/neu gene and centromere of chromosome 17 (CEP17) are used for FISH analysis.

Results

Representative slides of HER2 amplified CTCs by FISH analysis.
HER2+ on tissue biopsy, confirmed by LiquidScan



HER2- on tissue biopsy, HER2 amplified by LiquidScan



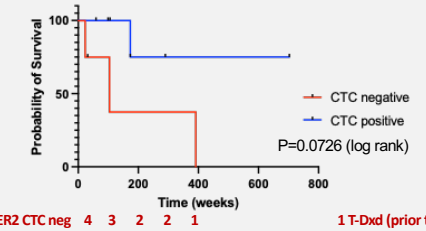
HER2 FISH positive per ASCO/CAP if:
- *HER2* copy number ≥6.0 signals/cell
- *HER2*/CEP17 ratio of ≥2.0
- *HER2*/CEP17 ratio of <2.0 with an average *HER2* copy number ≥6.0 signals/cell

Two HER2 amplified CTCs minimum required for sample to be HER2+

Nucleus: DAPI (blue)
HER2: TRITC (red)
CEP17: FITC (green)

Subgroups	Number (Percent)
HER2 Status by Tissue Biopsy	
$HER2_{null}$	6 (22.2%)
$HER2_{low}$	14 (51.8%)
HER2+	7 (25.9%)
Stage	
II-III	9 (33.3%)
IV	18 (66.7%)
HER2+ CTC detection rate by tissue HER2 status	
$HER2_{null}$ by biopsy	1 (16.7%)
$HER2_{low}$ by biopsy	8 (57.1%)
HER2+ by biopsy	6 (85.7%)
HER2+ CTC detection rate: localized vs metastatic	
II-III	6 (75%)
IV	8 (44.4%)

$HER2_{low}$ MBC Overall Survival



HER2 CTC neg 4 3 2 2 1 — 1 T-Dxd (prior to LiquidScan)
HER2 CTC pos 7 7 4 4 4 — 5 T-Dxd (1 prior to LiquidScan, 4 after)

Discussion

- We were able to demonstrate the use of a novel, non-invasive, CTC based method that could aid identification of HER2 amplification in patients with breast cancer.
- HER2 amplified CTCs were detectable in both localized and metastatic settings.
- HER amplification by CTC analysis compared favorably with that of tissue biopsy results for both HER2+ and $HER2_{null}$ breast cancer.
- A subset of patients with $HER2_{low}$ disease had detectable HER2 amplified CTCs. The presence vs absence of these CTCs may carry prognostic importance.

Conclusion

- To date, as a non-invasive approach to diagnosis, liquid biopsy has seen increasing utility in aiding cancer prognosis and therapeutic decision making. However, its role in clinical practice for breast cancer remains to be further explored.
- In our study, we demonstrated the feasibility of detecting HER2 amplified CTCs in blood samples of patients with biopsy-proven breast cancer, both in treatment naïve early stage disease as well as in the metastatic setting. We highlighted the potential prognostic role of the presence of HER2 amplified CTCs in the setting of $HER2_{low}$ breast cancer, though this study needs to be expanded to more patients.
- Future studies should additionally evaluate how HER2 amplified CTCs change over time with treatment. This would allow for elucidation on its role as a potential marker for therapeutic response.

References

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