Next Generation Liquid Biopsy (NGLB) mediated HER2+ amplification detection in patient samples.

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Abstract

Goal: Isolate and identify HER2+ breast cancer cells as a less invasive method for targeted therapies. (Pilot Project) LiquidScan™, an automated microfluidic liquid biopsy platform with high sensitivity and selectivity was used to isolate circulating tumor cells (CTCs) from whole blood of patients with HER2 amplified breast cancer (HER2+) and control patients (HER2-) that were diagnosed using conventional needle biopsies followed by FISH analysis. Circulating tumor cells (CTCs) were enriched using LiquidScan, followed by FISH diagnostics on the CTCs similar to the procedure performed on the needle biopsy samples. LiquidScan, is a fully automated Next Generation Liquid Biopsy system. Using this enrichment method high cellular CTC integrity by FISH analysis and 100% of all HER2+ patients as determined through pre-treatment needle biopsies were confirmed. In addition, 2 out of the 11 HER2- treatment naïve patients were found to have HER2+ CTCs after LiquidScan processing and **over 25% more patients could be identified as HER2+** (5 HER2+ patients by needle biopsies and 7 HER2+ patients by LiquidScan). Although the sample set was small (n=16), the results indicate that breast cancer needle biopsies performed even at stage 3 and 4 might misidentify HER2 status due to tumor heterogeneity and/or plasticity.

These findings are important for patient care since HER2-directed therapies may improve overall survival and outcomes in patients with HER2- breast cancer but with HER2+ CTC (3). The results for this pilot study indicate that LiquidScan can be used as a rapid analysis tool to evaluate HER2+ CTC status in patients with HER2- breast cancer on needle biopsies. Based on these results, further studies on larger patient sets and at earlier stages are planned.

Background: Overexpression of HER2 serves as an oncogenic driver in breast cancer which can result in focal progression and distant metastases. Amplification or overexpression of HER2 occurs in approximately 15–30% of breast cancers and serves as a therapeutic, prognostic, and predictive biomarker. With advances in HER2 targeting therapy, identifying patients who may benefit from these treatments, in addition to standard chemotherapy or endocrine therapy, are important. Rapid non-invasive monitoring for response to treatments are also needed. Liquid biopsy may provide a cost effective and sensitive approach to monitor treatment decisions and response.

Materials & Methods

Whole blood (6-8 ml) was collected from 16 consenting patients with confirmed stage 3 or 4 metastatic breast cancer with clinical HER2 status identified by solid tissue biopsy (5 HER2+ and 11 HER2-). CTCs were isolated with LiquidScan, probed by HER2 FISH, and analyzed using automated and visual microscopy.

The Liquid Biopsy System is designed to minimize biomarker loss (1). Many biomarkers are lost through sample handling from collection through processing. Three major sources for sample loss or alterations are: 1) Degradation, 2) Complex processing including sample prep 3) Interaction with instrument components. BioFluidica has developed a platform that minimizes these sources of biomarker loss. **Whole Blood** (or plasma for exosome isolation) is directly introduced into the microfluidic chip surfaced with antibodies designed for specific biomarker capture (2).

Figure 1. LiquidScan instrumentation has “Hamilton Inside” (First image). The blood sample tube is placed on the robot, and the operator can walk away. Chips (Third image) are housed on the LiquidScan module (second image), up to eight per run. Whole blood is introduced directly from the sample tube into the biomarker isolation chip. Blood is slowly injected (pushed) into one port on the chip and simultaneously withdrawn on the other port (a push-and-pull no loss system). The robotic system is scalable for fully automated processing. In this study, the microfluidic chip is surfaced with antibodies to specifically capture CTCs from whole blood.

Sixteen subjects were screened and enrolled at Moores Cancer Center, University of California, San Diego, CA, with a confirmed clinical diagnosis of stage 3 or stage 4 metastatic breast cancer; 5 patients were HER2 amplification positive and 11 were HER2 amplification negative. Eligible subjects must meet all of the inclusion criteria and none of the exclusion criteria listed below. Of 20 patients, 4 still need to be enrolled, leaving the total number of patients in this study as n=16.

•Inclusion Criteria

- Patients with a confirmed clinical diagnosis of stage 3 or stage 4 metastatic breast cancer.
- Patients who have had a successful solid tissue biopsy can provide positive (10 patients) or negative (10 patients) HER2 histopathology results within the last 12 months.
- Patients who are not currently receiving an active chemotherapeutic agent.
- Patients can travel to the study site and provide 9 mL of blood by standard venipuncture.
- Females ≥ 18 years of age, as of the enrollment date. There is no upper age limit for donor enrollment.
- Patients who can provide informed consent.

All patients were tested for HER2 status using needle biopsies followed by HER2 fluorescence in situ hybridization (FISH).

Sample Collection: Each patient donated 6-8 ml of blood collected by venipuncture. The blood was collected in a BioFluidica Blood Collection Tube, a special tube that preserves cell viability and minimizes cell aggregation and micro clotting during transport for optimized microfluidics applications in Biofluidica sinusoidal channel chips.

LiquidScan: CTCs were isolated from whole blood without preprocessing. A low flow rate through 150 parallel sinusoidal microchannels allows rapid processing (2 hrs), where CTCs from one patient are collected in one tube, ready for analysis.

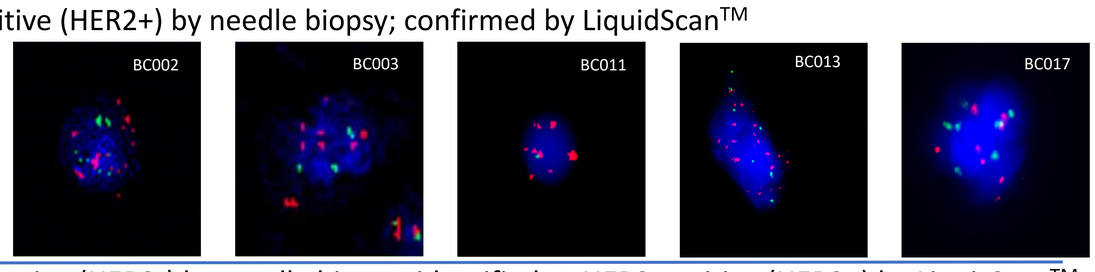
FISH Analysis: Isolated and enriched CTCs were eluted from the microchip then deposited on a slide using cytospin and analyzed with specific reagent probes for HER2 (Texas Red) and centromere 17 (FITC).

Results

	HER2 Status			
Patient ID	**Needle Biopsy**	**LiquidScan**	**Stage**	**Cellularity**
BC002	HR+, HER2+	HER2+	Stage 4	Good
BC003	HR+, HER2+	HER2+	Stage 3	Excellent
BC011	HR-, HER2+	HER2+	Stage 3	Excellent
BC013	HR-, HER2+	HER2+	Stage 3	Excellent
BC017	HER2+	HER2+	Stage 4	Scant
BC004	TNBC	HER2-	Stage 4	Good
BC005	TNBC	HER2-	Stage 4	Excellent
BC006	TNBC	HER2-	Stage 3 or 4	Excellent
BC007	TNBC	HER2-	Stage 3	Excellent
BC008	TNBC	HER2-	Stage 4	Excellent
BC009	HR+, HER2-	HER2-	Stage 4	Excellent
BC010	TNBC	HER2-	Stage 3	Excellent
BC014	TNBC	HER2-	Stage 4	Excellent
BC015	TNBC	HER2-	Stage 4	Excellent
BC012	TNBC	**HER2+**	Stage 3	Excellent
BC016	HR+/HER2-	**HER2+**	Stage 4	Excellent

Table 1. The 20-patient pilot study was completed for 16 patients. All patients had needle biopsies to determine their HER2 status. Patients with HER2 positive biomarkers were eligible for targeted Anti-HER2 therapy. All patients donated one tube of blood for positive CTC selection using LiquidScan.

HER2positive (HER2+) by needle biopsy; confirmed by LiquidScan™



HER2-negative (HER2-) by needle biopsy; identified as HER2 positive (HER2+) by LiquisScan™

- Nucleus: DAPI (Blue)
- HER2:TRITC (Red)
- CEP17: FITC (Green)

Figure 2. Row 1: HER2+ circulating tumor cells isolated from breast cancer patient samples. BC002, BC003, BC011, BC013, and BC017 were identified as HER2+ Breast Cancer by needle biopsy prior to sample collection. Although this is a small sample set, we were able to demonstrate that non-invasive liquid biopsy with the LiquidScan identifies HER2 status with high concordance with conventional solid biopsy. **Row 2:** HER2+ CTCs were identified in patient samples BC012 and BC016 identified by needle biopsy as HER2 negative. With advances in HER2+ breast cancer management, there is a need to identify patients with HER2+ early breast cancer who may benefit from de-escalation or escalation of treatment and liquid biopsy may provide a cost effective, sensitive, and non-invasive approach to monitor response.

Discussion

We were able to demonstrate that the use of a new non-invasive liquid biopsy methodology could lead to rapid and accurate identification of HER2 status in patient samples. The results of this study compared more than favorably with the results drawn from needle biopsy. This study demonstrated two patients as HER2+ whereas diagnosed by needle biopsy as HER2-. While we plan to conduct a larger CTC breast cancer study, the broader use of LiquidScan strongly indicates the importance of being able to perform liquid biopsy at the cellular level.

Conclusions

As a non-invasive approach to diagnosis, liquid biopsy has seen a meteoric rise in the number of tests and their acceptance, most tests being based on analysis of cell-free DNA (cfDNA) present in blood. Notwithstanding the importance of cfDNA-based tests, many diseases and genetic environments are not amenable to a cfDNA test, whether through the size of the DNA marker, the relative concentration of the marker in blood, or a need to explore more within the biology of the disease in question, the importance of being able to analyze at the cellular level has not been lost. **The BioFluidica LiquidScan technology provides a next-generation liquid biopsy in which sensitive and accurate analyses can be performed at the cellular level (4).**

Reference

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