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, a no-loss microfluidics isolation & enrichment system maintained high cellular integrity by FISH analysis on the isolated CTCs and confirmed 100% of all HER2+ patients as determined through needle biopsies. In addition, 2 out of the 11 HER2- patients were identified as HER2+ after LiquidScan CTC enrichment. Although the sample set was small (n=16), the results indicate that breast cancer needle biopsies performed even at stage 3 and 4 breast might be misidentified due to tumor heterogeneity. This finding is even more important for patient care since HER2 positivity directs personalized treatments that are highly successful for positive disease control.

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. Patients have poor response to standard chemotherapy regimen but several Anti-HER2 therapies are commonly recommended. Even with these therapies, researchers have found that 10% to 23% of women diagnosed with small, HER2-positive cancer recur within 5 years compared to ~5% of women with HER2-negative cancer. Rapid monitoring tests for response to treatments are needed. Liquid biopsy may provide a cost effective and sensitive approach to monitor patients' 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. 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.

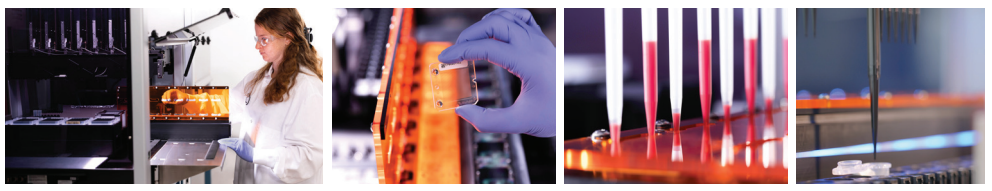

Figure 1. LiquidScan instrumentation has "Hamilton Inside" (First image). The microfluidic chips surfaced with antibodies to capture CTCs are placed on the Microfluidic Chip Processing Module (MCPM) which is slotted into the liquid handling robot (Images 1 and 2). Once whole blood sample tubes are placed on the robot, and the run software initiated, the operator can walk away. Blood is introduced directly from the sample tube into the chip by slowly dispensing into one port on the chip and simultaneously aspirating the other port (two-pipette tips per chip – a closed loop no-loss system (Image Three). After washing, captured cells are released from the chip and eluted into assay tubes for downstream analysis (Image 4).

Sixteen subjects were screened and enrolled at Moores Cancer Center, the 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 taking 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.

Exclusion Criteria

- ✔ Patients with known bleeding disorders
- ✔ Pregnant or nursing females
- ✔ Patients with known HIV, HTLV, or Hepatitis infection

All patients were tested for HER2 status using needle biopsies followed by HER2 fluorescent 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).

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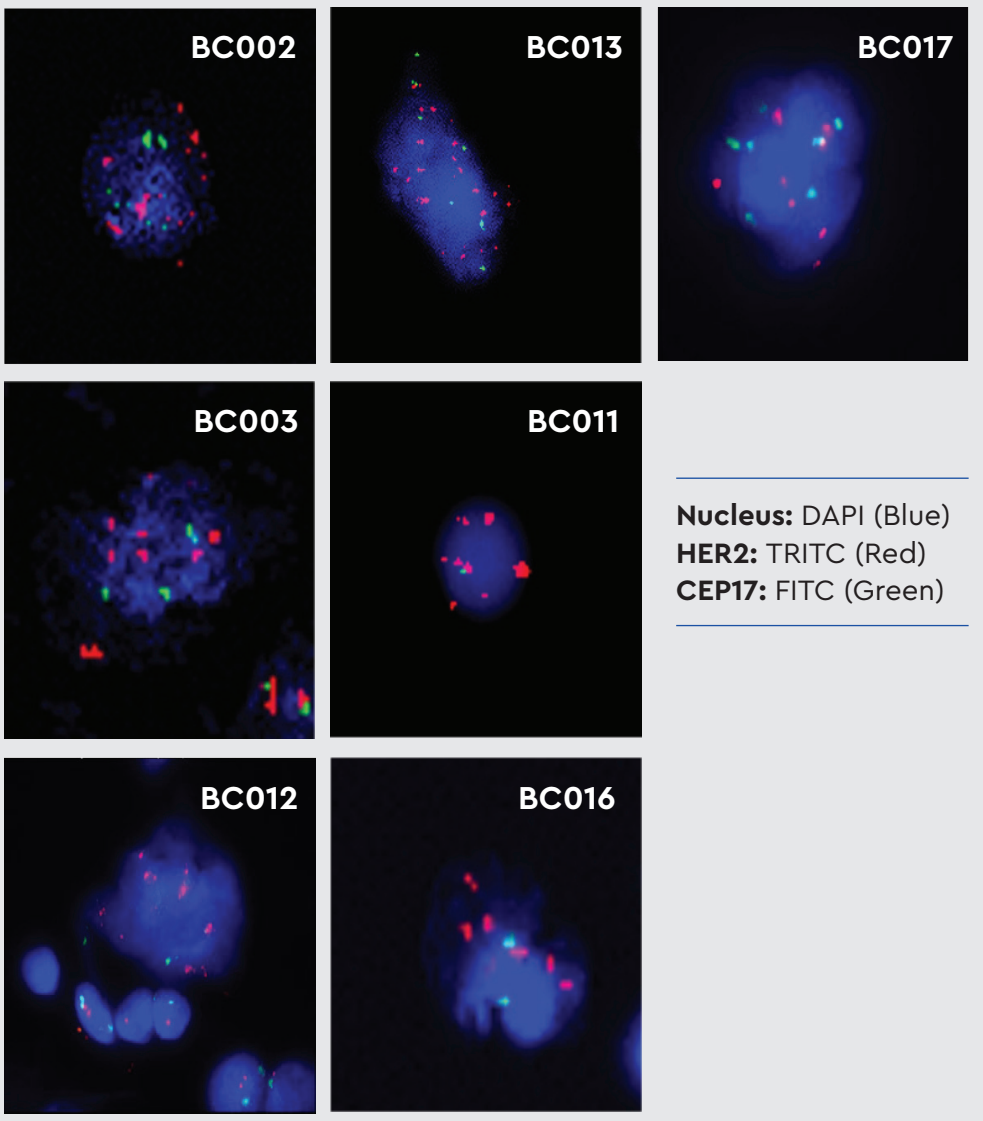
RESULTS

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.



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 surgical biopsy. This study demonstrated two patients as HER2+ whereas diagnosed by surgical biopsy as HER2-. While we plan to conduct a larger CTC breast cancer study, the broader use of LiquidScan (see References below) 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 (LB) 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 (NGLB) in which sensitive and accurate analyses can be performed at the cellular level.**

REFERENCE

1. Discrete microfluidics for the isolation of circulating tumor cell subpopulations targeting fibroblast activation protein alpha and epithelial cell adhesion molecule. Witek MA, Aufforth RD, Wang H, Kamande JW, Jackson JM, Pullagurla SR, Hupert ML, Usary J, Wysham WZ, Hilliard D, Montgomery S, Bae-Jump V, Carey LA, Gehrig PA, Milowsky MI, Perou CM, Soper JT, Whang YE, Yeh JJ, Martin G, Soper SA. NPJ Precis Oncol. 2017;1:24. doi: 10.1038/s41698-017-0028-8. Epub 2017 Jul 25. PMID: 29657983; PMCID: PMC5871807