Isolation and analysis of pure intact tumor cell populations from FFPE: implications for more precise HER2 FISH testing in breast cancer

Amanda Gerber(1), Trisiky C. Clarin (1), Ambica Bhandari (3), Chiara Bolognesi (2), ., Nicolò Manaresi (2) Gianni Medoro (2), Mathew Moore(3), Philip D. Cotter (3) and Farideh Z. Bischoff (1)
(1) Menarini Silicon Biosystems Inc, San Diego, CA (2) Menarini Silicon Biosystems, Bologna, Italy (3) Research Dx, Irvine, CA

Introduction
Guidelines worldwide focus on the importance of precise, reproducible, and quality assurance of fluorescent in situ hybridization (FISH) methods for testing companion diagnostic markers, including Human Epidermal Growth Factor Receptor 2 (HER2) gene amplification in breast cancer. Despite these guidelines, variations in test results due to pre-analytical sampling and tissue processing are observed. Accurate enumeration and characterization of these cells can be useful for ascertaining prognosis. Evaluation of more precise HER2 status can provide valuable information for consideration of therapy in patients. The DEPArray™ platform demonstrates reliability in isolating pure populations of cells from complex tissues and the concurrent recovery of 100% pure tumor cell populations. This allows for a unique approach to isolate pure and intact tumor cells. In this study, we report the utility of the DEPArray™ platform for precise subsequent downstream FISH analysis from breast cancer formalin-fixed, paraffin-embedded (FFPE) samples.

Methods
Fifty-micron thick FFPE sections originating from breast cancer tumors (n=7; each with a reported HER2/CEP17 ratio) and positive control SK-BR3 breast cancer cells were disaggregated down to single cell suspensions and stained with cytokeratin, vimentin and DAPI. Isolation of pure and intact cytokeratin-positive/vimentin-negative/DAPI positive tumor cells (mean=180, range from 107 to 300) from each sample was achieved using the DEPArray™ platform, an automated system enabling image-based cell sorting with single-cell resolution. Recovered cells were then cytospun onto poly-L-lysine-coated glass slides and washed in 100% EtOH allowing for pure tumor cell FISH analysis. The isolated tumor cells were processed for interphase FISH for HER2 gene amplification using the PathVysion DNA Probe Kit (Abbott PathVysion/Vysis) specific to the centromere of chromosomes 17 (CEP 17 Spectrum Green) and the locus-specific HER2 probe (Spectrum Orange). Scoring of the signals was performed on 20 pure tumor cell nuclei. A ratio of HER2/CEP17 >2 in cell nuclei was regarded as positive for HER2 gene amplification.

Results

- FFPE Breast Cancer samples from 7 patients and FFPE SK-BR3 positive control were studied.
- Among the patient samples, 25.5% of the DEPArray™ isolated tumor cells were recovered onto slides prior to FISH.
- Positive HER2 amplification levels for the FFPE derived control SK-BR3 cells were observed (HER2/CEP17 ratio >4.4) and consistent with levels reported in the literature.
- DEPArray HER2 status as determined for each of the patient samples reported to be positive or negative by FISH was consistent with the pathology results. Moreover, we observe that the HER2 ratios for the HER2 negative cases now approach the expected 1.0 ratio.
- More precise determination of HER2 status is demonstrated in the two HER2 IHC+2 cases; both becoming HER2 negative only after analysis of pure tumor cells.

Conclusions
We demonstrate feasibility in performing FISH for HER2/CEP17 on pure and intact tumor cells isolated from breast cancer derived FFPE using the DEPArray™ platform. Using a 50 µm FISH section allowed recovery of whole, intact, tumor cells based on immunostaining for cytokeratin, vimentin, and DAPI. Efficient recovery of the DEPArray™ sorted cells onto slides further permitted routine FISH analysis of individual isolated tumor cells. These preliminary results imply the possibility of the DEPArray™ platform bringing digital precision to detection, quantification and recovery of pure target cells for subsequent FISH downstream analysis when standard FISH results are inconclusive or when insufficient tumor content prohibits downstream analysis. Analytical validation to evaluate the implications of accurate and precise single cell HER2 enumeration using the DEPArray™ platform is underway with larger numbers of patient FFPE breast cancer samples.