

Implementation of a novel spectral flow cytometry-based theragnostic test for circulating tumor cells in breast cancer

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Abstract

Background: Despite successful surgical management, breast cancer (BC) can recur many years post-diagnosis, indicating the potential dormancy of cancer cells in distant sites. Circulating tumor cells (CTCs), present in peripheral blood (PB) or pleural effusions (PE), serve as biomarkers for minimal residual disease (MRD). The presence of single CTCs or clusters of CTCs with white blood cells (CTC-WBC) correlates with heightened metastatic potential. Notably, CTC characteristics display significant heterogeneity both intra- and inter-individually among patients, even within the same BC subtype.

Objective: The detection and phenotyping of CTCs are critical for diagnosing MRD and guiding patient-specific therapeutic strategies based on theragnostic markers.

Methods: We established an easily integrable spectral flow cytometry (SFC) panel to detect multiple markers simultaneously at the single-cell level, allowing for detailed characterization of CTCs in BC patients. This panel includes markers delineating BC subtype, epithelial and mesenchymal traits, theragnostic indicators, and CTC-WBC clusters.

We applied this panel to characterize CTCs in PE samples from metastatic BC (MBC) patients, with non-tumor patients serving as controls.

Results: In silico analysis and single-cell RNA sequencing of sorted CTCs from BC patients facilitated the design of an antibody panel encompassing 33 markers. The specificities of these antibodies were validated on various cancer

cell lines. The panel was tested on PE samples from 21 MBC patients and controls, assessing CTC detection quantitatively via a 5-laser SFC instrument (Cytek Aurora). PE was preferred due to its higher frequency of CTCs and CTC-WBC cluster

s compared to PB. Unsupervised data analysis successfully differentiated single CTCs, CTC-WBC clusters, and immune or mesothelial cells, revealing significant intra- and inter-individual heterogeneity in CTC marker expression.

Additionally, imaging flow cytometry confirmed the presence of CTC-WBC clusters.

Conclusion: Our SFC panel enables the characterization of diverse CTC phenotypes in the PEs of MBC patients, underscoring the heterogeneity of CTCs and providing insights for prognostic and therapeutic applications.

Do you have any conflicts of interest?

No, I do not have a conflict of interest.