Liquid biopsy of intact CTCs for longitudinal, actionable detection of early changes in breast cancer, stages I-IV

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Background

From early in their development, tumors release circulating tumor cells (CTC) in the bloodstream. CTCs have a short half-life in circulation and express diverse proteomic profiles, since they originate from genetically heterogeneous malignant tissue. *QCDx-br™* is an enrichment-free, liquid biopsy (LB) designed to detect intact/live CTCs, with high sensitivity, by mapping Epithelial-to-Mesenchymal status and expression of actionable biomarkers indicating potential for anti-HER2, endocrine, and triple-negative treatments, and inform the oncologist on potential, timely intervention both in early and metastatic breast cancer (BC).

Methods

QCDx-br was validated in an observational, prospective and blinded trial with 11 neoadjuvant (Stage I & II) and 22 metastatic (Stage IV) BC patients, recruited under a protocol approved by the University of Connecticut Cancer Center Institutional Review Board. Longitudinal blood samples were drawn from each patient at 8-10 timepoints over a 24-month period and processed with *QCDx-br*. Preparations of intact WBCs (>1 million cells) were stained by immunofluorescence (IF), immobilized in hydrogel and imaged automatically with Fluorescence Light Sheet Microscopy (FLSM). 3D-images with subcellular resolution were processed with proprietary cell segmentation and classification algorithms for profiling CTCs phenotypically and to detect actionable IF targets.

Results

CTCs were detected in >85% of tested samples from neoadjuvant and metastatic patients. In neoadjuvant patients, CTCs expressing actionable markers were seen to gradually decline in response to treatment. In the metastatic cohort, changes in CTC profiles and burden provided intelligence on the patient's response to treatment. Actionable markers were seen in CTCs before they were detected by tissue biopsy. Patients in ongoing therapy after metastatic disease diagnosis had negative imaging for several years before they were enrolled, to assess disease relapse with *QCDx-br*. No CTCs were seen in a matched population of healthy donors.

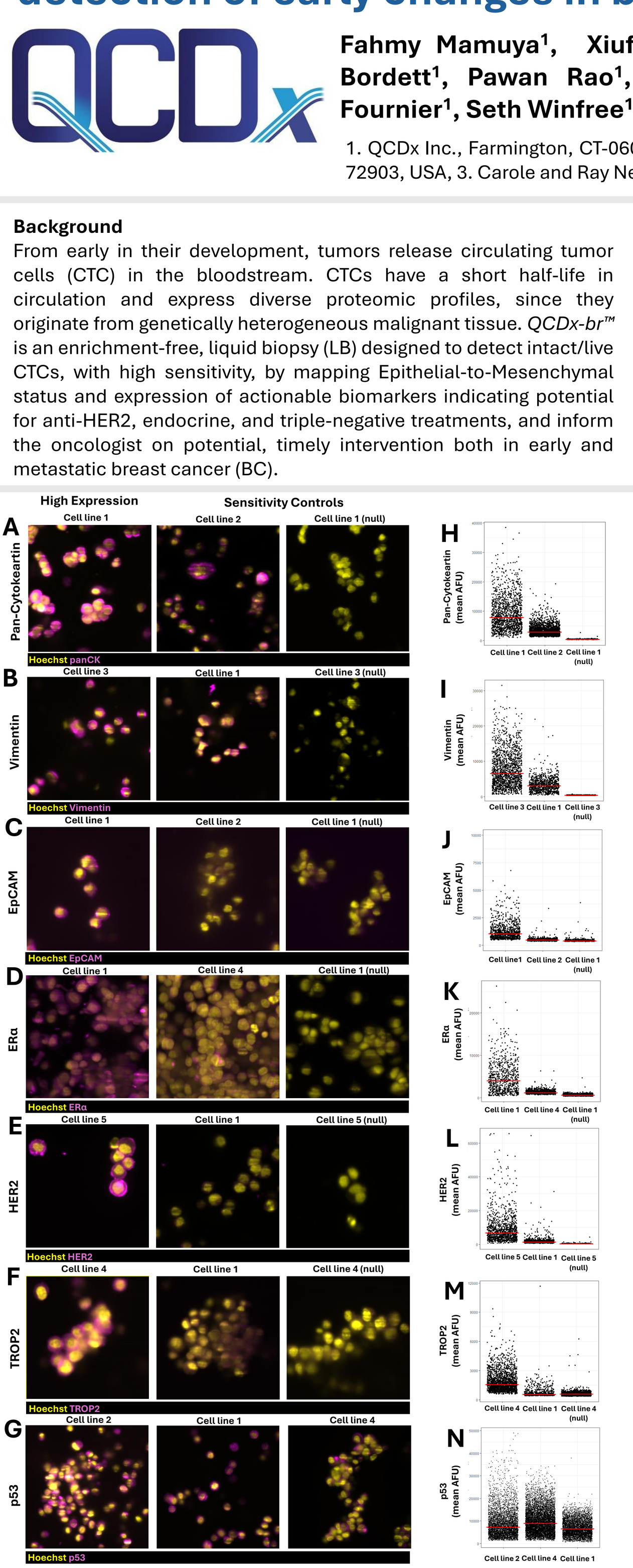

Figure 1. Establishing a quantitative CTC and therapeutically relevant immunofluorescence panel. To verify and validate antibodies for identification of CTCs and quantitate therapeutically relevant proteins in breast cancer, five cell lines expressing varying levels of Cytokeratin, Vimentin, EpCAM, HER2, ERα, TROP2 or p53 were stained in suspension with antibodies against these markers and Hoechst, mounted in hydrogel and imaged on a light sheet fluorescence microscope in 3D (A-G). The 3D volumes were segmented in all channels and the mean arbitrary fluorescence unit (AFU) measured per cell and plotted (H-N). For comparison a negative control, no antibody (null), or low-expression was included (A-F and H-M). Vimentin, pan-Cytokeratin and EpCAM are mixed for a "CTC marker." The median is indicated by red line.

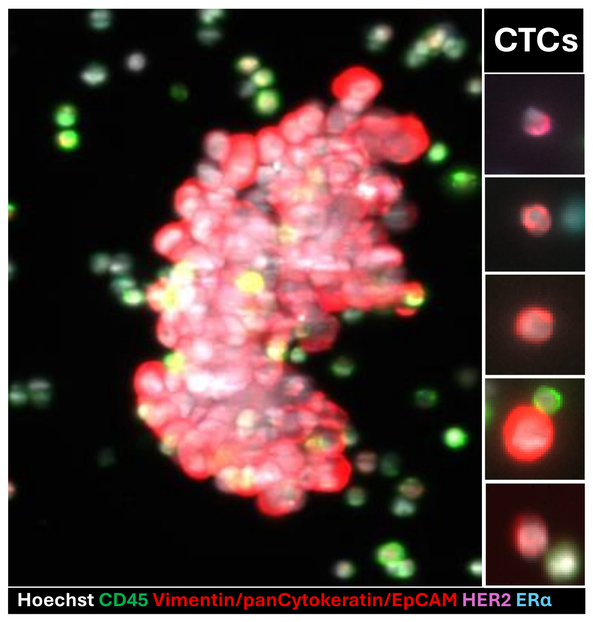

Figure 2. Detection of CTCs and therapeutically relevant markers. More than 250 million cells have been imaged in 3D and processed of which ~30 million have been screened automatically and by two levels of human subject experts identifying 214 CTCs. CTCs have been detected in both the neoadjuvant and metastatic cohorts either alone or in large cellular aggregates (right and insets).

Cohort	Stage	Total CTCs	ERα-positive	HER2-positive	Total verified (cells)	Outstanding (cells)
Neoadjuvant	I-II	55	31	-	8,081,096	68,168,904
Metastatic	IV	159	107	51	25,126,760	139,873,240
Healthy Donors	NA	ND	ND	ND	-	12,500,000

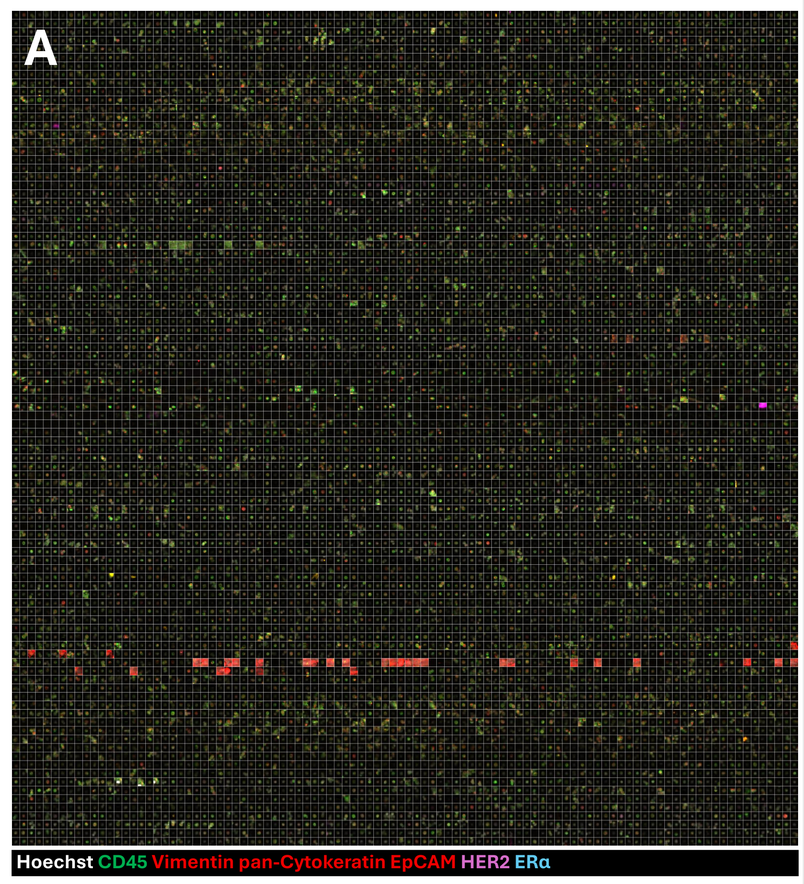

Figure 3. Multiplex phenotyping of > $5x10^8$ cells for big data at cellular resolution in liquid biopsies. A. Random sampling of 9800 segmented cells in the CLINBREAC cohort as maximum projections stained with Hoechst, CD45, Vimentin, pan-Cytokeratin, EpCAM, HER2, and ERα. High and moderate levels of the CTC markers are identifiable in this random selection (Red). **B.** Total cells in dataset; analysis to be completed by EOY 2025. **C.** MultiFluor: next-gen cyclic IF on suspension cells.

B

Cohort	Dataset (estimated cells)
Neoadjuvant	152,500,000
Metastatic	330,000,000
Healthy Donors	25,000,000

Conclusion

The CLINBREAC breast cancer study indicates that *QCDx-br™* LB combined with FLSM microscopy enables sensitive identification of CTCs expressing actionable therapeutic targets to determine best treatments in BC Stages I-IV. It indicates evidence for real-time evaluation of treatment responses in both early and advanced disease and may have the potential to improve early disease detection, including minimal residual disease.

Conflict of Interest Declaration

F.M., X.L., E.L., P.R., D.F., S.W. and T.T. are employees of QCDx Inc.
A.R., A.A.S. and S.T. declare no conflict of interest