TROP2 and HER2 Expression by Liquid Biopsy in Women with Metastatic Triple Negative Breast Cancer

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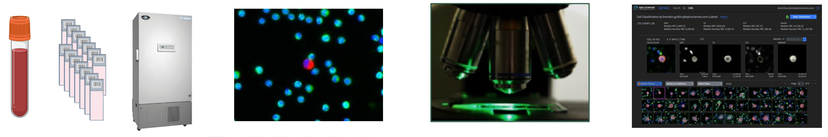
BACKGROUND

- Blood based biopsy can inform treatment decisions for metastatic Breast Cancer (MBC) patients when conventional tissue biopsies are not feasible.
- Tissue biopsy may not be representative of biologically heterogeneous sites of metastatic disease.
- Trophoblast cell-surface antigen 2 (TROP2) and HER2 protein are prominent in metastatic cancers, including metastatic triple-negative breast cancer (mTNBC), which has limited treatment options.
- TROP2 and HER2 antibody-drug conjugate targeted therapies have demonstrated high efficacy for MBC patients, including patients with low levels of each biomarker.
- We demonstrate the technical feasibility and proof of concept of a new liquid biopsy TROP2 assay together with an established HER2 assay for measurement of Circulating Tumor Cell biomarkers mTNBC patients.

METHODS

EPIC SCIENCES CTC DETECTION PLATFORM

1. Slide Preparation
2. Cell Staining
3. Scanning
4. Single cell digital pathology



CK, CD45, DAPI, Biomarker

Figure 1: Schematic of CTC collection and detection process: **1)** Nucleated cell from blood are plated onto slides and stored in the -80˚C biorepository; **2)** Slides are stained for DNA (DAPI), CK, CD45, and TROP2; **3)** Slides are scanned; **4)** CTC candidates [DAPI$^{(+)}$, CK$^{(+)}$, CD45$^{(-)}$] detected by multi-parametric digital pathology algorithm.

RESULTS

Selection of Cultured Cell Lines to Demonstrate IF Assay Dynamic Range

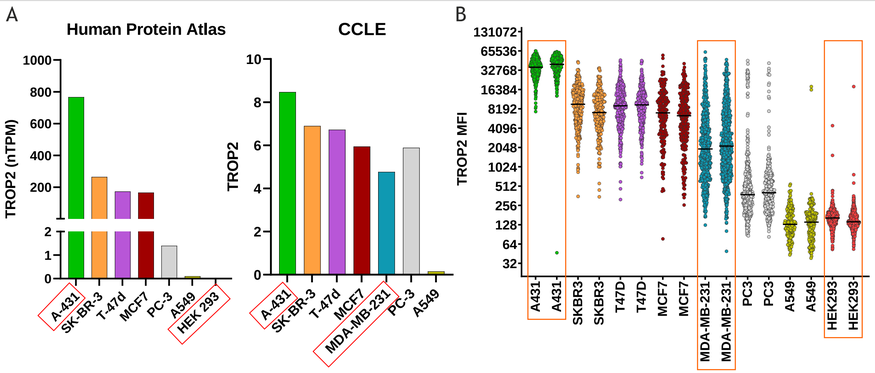


Figure 2: Selection of cultured cell lines to demonstrate IF assay dynamic range. **(A)** RNA-Seq data for cell lines tested during TROP2 assay development extrapolated from Human Protein Atlas database and Cancer Cell Line Encyclopedia (CCLE) database. **(B)** TROP2 specific antibody was tested in eight cell lines with different TROP2 expression levels which follow the same trend demonstrated with RNA-Seq data. Three of these cell lines (highlighted in the square boxes) were selected for use during development of the TROP2 assay.

Representative Immunofluorescent (IF) Images from Control Cell Lines

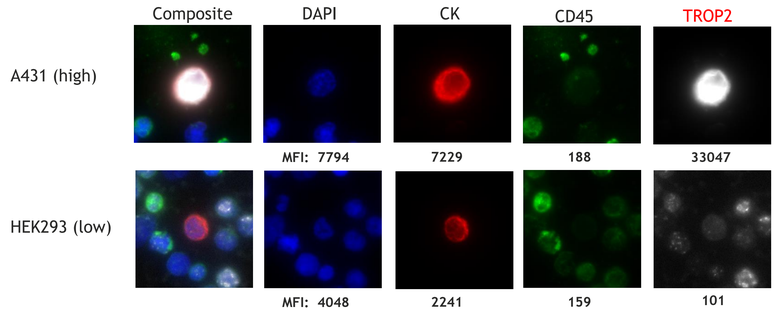


Figure 3: TROP2 expression in A431 and HEK293 cell lines. TROP2 signal (MFI) was detected on tumor cell lines [DAPI$^{(+)}$, CK$^{(+)}$, CD45$^{(-)}$].

TROP2 IF Signal Across Control Cell Lines and Patient WBCs

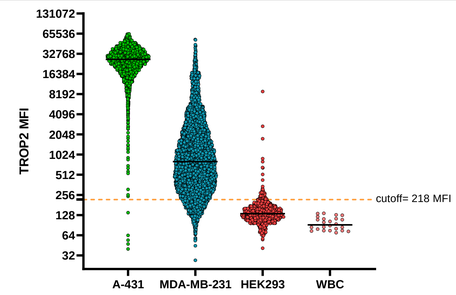


Figure 4: TROP2 expression in low (HEK293), medium (MDA-MB-231), and high-expressing (A-431) cell lines as well as in White Blood Cells (WBC). Each dot on the plot is an individual CLC from a control cell line. For WBC, each dot represents the median TROP2 IF signal across millions of WBCs per each slide. Threshold of positivity (analytical cutoff) of 218 MFI was set based on a 95% confidence level of the low expressing cell line.

Representative IF Images from mTNBC Patients

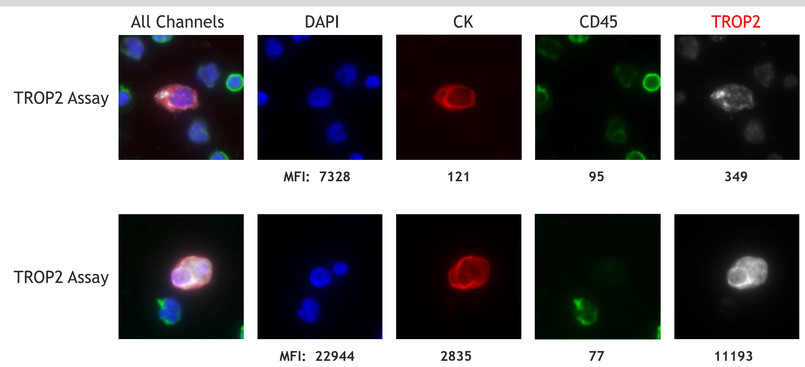
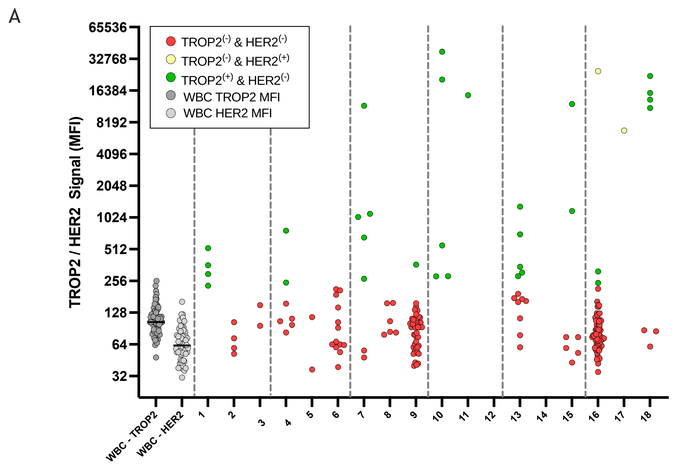


Figure 5: TROP2 expression in mTNBC patients. CTC are defined as DAPI$^{(+)}$, CK$^{(+)}$, and CD45$^{(-)}$. Top row, a single TROP2$^{(+)}$ CTC. Bottom row, a cluster of two distinct TROP2$^{(+)}$ CTCs.

TROP2 and HER2 IF Assay Signals in mTNBC Patient Samples



18 mTNBC Patient Samples Stained with Epic Sciences TROP2 and HER2 Assays

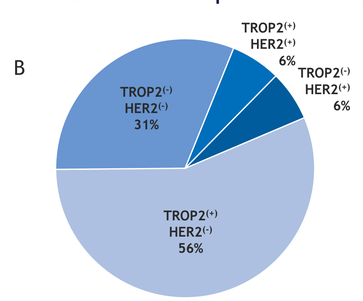


Figure 6: Assessment of TROP2 and HER2 expression among 18 mTNBC samples.

89% of patients (16 patients) with mTNBC had detectable CTCs. (A) Each dot on the plot is an individual CTC from a patient. TROP2 MFI, and HER2 MFI across all cells had a wide dynamic range (mean:1357, range: 37-38281, and mean:634, range: 35-24985, respectively). (B) TROP2 and HER2 positivity proportions within patients with CTCs.

CONCLUSIONS

- We report on developing a TROP2 immunofluorescent assay that utilizes Epic Sciences' CTC detection platform.
- Across exploratory mTNBC patients, a high prevalence (89%) of TROP2 expressing CTCs was detected with a wide dynamic range in biomarker signal.
- Blood-based assessment of mTNBC patients could further characterize relevant cancer phenotypes and may provide additional information for decision-making for anti-TROP2-antibody conjugate drugs (ADC) based on their mechanisms of action.