Circulating Tumor Cell (CTC) Detection and 5T4 Characterization in Breast, Ovarian, and Lung Cancer Patients on Active Therapy

Abstract # 565

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Introduction

Monitoring CTCs from a blood draw could enable a minimally invasive, longitudinal observation of an evolving disease[1]. Antibody drug conjugates (ADC) are a promising approach to deliver highly potent chemotherapeutics to cells expressing target proteins[2]. An ADC that targets 5T4, an oncofetal antigen expressed on tumor initiating cells[3], is currently in clinical development. Patients with tumors or CTCs expressing 5T4 may benefit from this therapeutic[4]. We sought to develop a 5T4 CTC test and to evaluate the incidence of 5T4 expressing CTCs in breast, ovarian, and lung cancer patients.

Materials and Methods

Cell Lines: Three control cell lines with varying 5T4 expression were used (NCI-H226: high, NCI-H1975: medium, and NCI-H460: low-negative) for assay development.

Patient Samples: Blood samples from breast (n=10), ovarian (n=9) and NSCLC (n=25) patients on active therapy were collected prospectively (ConversantBio Huntsville, AL) and shipped to Epic Sciences for analysis. All samples were appropriately consented. Each sample was accompanied by a pathology report giving details of indication.

Epic CTC Platform: (1) Nucleated cells from blood sample were placed onto slides and stored at -80C. (2) Slides were stained with Cytokeratin (CK), CD45, DAPI and 5T4 (utilizing a monoclonal antibody). (3) Slides were scanned in 4 channels (4) Pathology algorithm identified CTC candidates confirmed by human reader. (5) 5T4 characterized on each CTC (Figure 1)

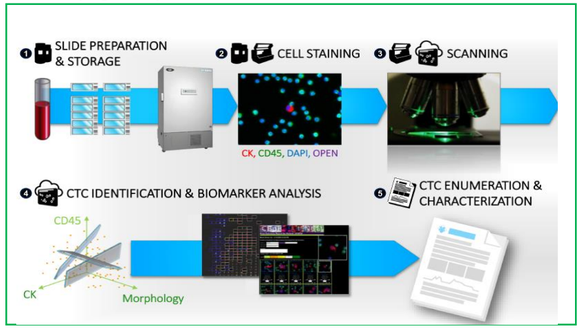


Figure 1: Schematic of Epic CTC Platform

Results

Assay Development:

A novel CTC assay utilizing a monoclonal antibody against 5T4 was developed. Three cell lines with varying 5T4 expression were spiked into normal whole blood to assess the linearity of CTC detection. These cell lines as well as 5T4 high and 5T4 low patient samples (previously assessed) were used to assess intra- and inter-assay reproducibility of % 5T4(+) CTCs.

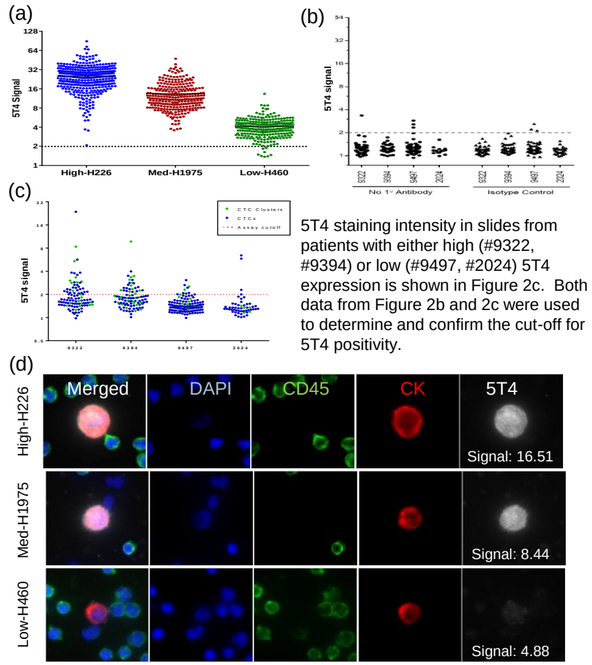
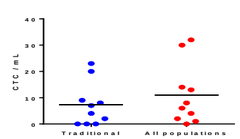


Figure 2: (a) Dot plot of 5T4 signal of cell lines (b) Dot plot of 5T4 signal (stained with no 1° Ab or isotype control) from patient samples in clinical feasibility (CF) study (c) 5T4 signal for 4 patients in CF study (d) Representative 40x images of 5T4 staining for 3 cell lines

5T4 staining intensity in slides from patients with either high (#9322, #9394) or low (#9497, #2024) 5T4 expression is shown in Figure 2c. Both data from Figure 2b and 2c were used to determine and confirm the cut-off for 5T4 positivity.

Patient Samples

Breast Cancer: 8 of 10 patients had traditional CTCs (CTC + CTC cluster) defined by CK positivity. 9 of 10 patients had CTCs when additional CTC subpopulations (CK- CTCs, and apoptotic CTCs) were considered. The mean CTC count for the 10 patients examined was 11 CTC/mL with all subpopulations (Figure 3). 5T4 expression varied among the breast cancer patients (Figure 4), with the highest % positivity seen in patients with corresponding ER(+) primary tumors. The mean 5T4 burden (calculated by adding the 5T4 intensity value for each CTC within a sample and divided by the # of CTCs in that sample) was 26.3.



Summary Statistics		
	Traditional (CTC/mL)	All Populations (CTC/mL)
Mean	7	11
Median	6	7
Range	0 – 23	0 – 32

Figure 3: CTC/mL count for breast cancer patients

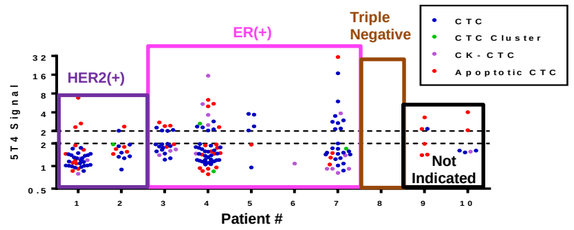


Figure 4: Detection of 5T4+ CTCs in 8 of 10 breast cancer patients

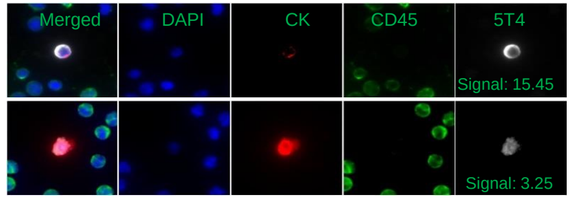
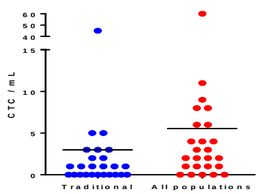


Figure 5: Representative images of 5T4+/CK- (top) and 5T4+/CK+ (bottom) CTCs from Breast Cancer patients

NSCLC:14 of 25 patients had traditional CTCs. 21 of 25 patients had CTCs when additional subpopulations were considered. The mean CTC count for the 25 patients examined was 6 CTC/mL with all subpopulations (Figure 5). 5T4 expression varied among the lung cancer patients (Figure 6). The mean 5T4 burden was 39.1 and was observed to be the highest among the three disease indications examined in this study.



Summary Statistics		
	Traditional (CTC/mL)	All Populations (CTC/mL)
Mean	3	6
Median	1	2
Range	0 – 45	0 – 60

Figure 6: CTC/mL count for NSCLC patients

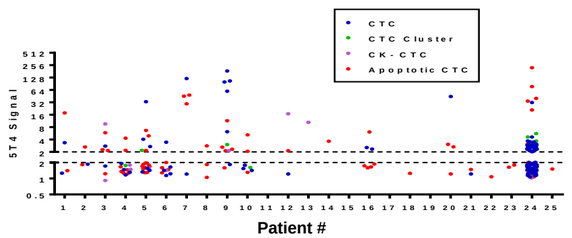


Figure 7: Detection of 5T4+ CTCs in 21 of 25 NSCLC patients

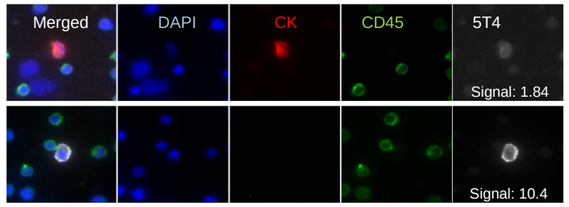


Figure 8: Representative images of 5T4-/CK+ (top) and 5T4+/CK- (bottom) CTCs from NSCLC patients

Ovarian Cancer: 2 of 9 patients had traditional CTCs. 3 of 9 patients had CTCs when additional subpopulations were considered. The mean CTC count for the 9 patients examined was 0.1 CTC/mL with all subpopulations (Figure 8). No 5T4 positive CTCs were detected in these 9 Ovarian cancer patients. The mean 5T4 burden was 1.

Summary Statistics		
	Traditional (CTC/mL)	All Populations (CTC/mL)
Mean	0.1	0.5
Median	0	0
Range	0 – 0.6	0 – 3.6

Figure 9: CTC/mL count for Ovarian cancer patients

Conclusions

- 5T4 expressing CTCs were detected in the blood of NSCLC and breast cancer patients who have received standard of care therapy.
  - A higher percentage of breast cancer patients (80%) had CTCs than NSCLC (56%) or ovarian patients (22%)
  - The number of traditional CTC/mL was greater in breast cancer patients (7.4 CTC/mL) than in NSCLC (2.8 CTC/mL) or ovarian cancer patients (0.1 CTC/mL)
  - The 5T4 expression was higher in NSCLC patients (mean 5T4 burden of 39.1) than in breast cancer (mean 5T4 burden of 26.3) or ovarian (mean 5T4 burden of 1) cancer patients
- This assay can be deployed in upcoming clinical trials to enumerate and characterize 5T4 expressing CTCs as
  - PD/PK biomarkers
  - Predictive biomarkers

References

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