Real-time monitoring of CTC number, biomarker expression, and kinetics as prognostic and predictive markers in patients with GI malignancies

Erin Bayer[1], Brock M. Bartels[1], Rachel N. Ponting[1], Areeb Lutfi[2], Maaz K. Afghan[2], Arturo B. Ramirez[1], Pashtoon M. Kasi[2]

[1]RareCyte, Inc., Seattle, WA. [2] Division of Hematology and Medical Oncology, Department of Medicine, Weill Cornell Medicine, Cornell University, New York, NY.

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BACKGROUND

Circulating Tumor Cell (CTC) analysis provides non-invasive, rapid insight into tumor burden and early assessment of patient therapeutic response. Importantly, it also provides quantitative expression of both prognostic and predictive protein biomarkers. ctDNA analysis also provides valuable insight into therapeutic targets in addition to mutational burden, but the speed and cost of analysis are prohibitive, especially when it comes to serial testing. Using a cohort of patients with advanced or metastatic gastrointestinal (GI) cancers, we herein show the feasibility of using CTC enumeration, biomarker analysis, and kinetics as a complementary metric or alternative to ctDNA testing.

METHODS

Using the RareCyte platform, nucleated cells from blood were collected, spread on slides, stained, and imaged with CyteFinder® for CTC identification (CK/EpCAM+, CD45-) and quantification of biomarkers (PD-L1/HER2 or Ki67/EGFR). CTC enumeration (CLIA-accredited CTC test) and biomarker status for each patient were tracked over time and compared with ctDNA mutational analysis and tumor burden.

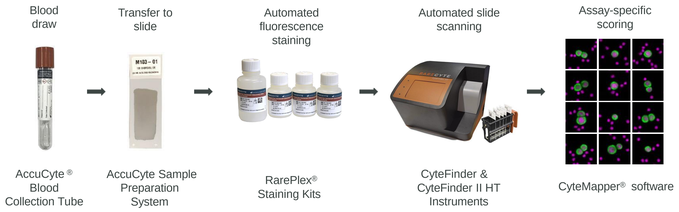

Figure 1. RareCyte platform workflow. Blood is collected into AccuCyte Blood Collection Tubes (BCTs). Nucleated blood cells are processed to slides using the AccuCyte Sample Preparation System. Slides are stained with assay-specific RarePlex staining kits. Slides are scanned using the CyteFinder Instrument and images are analyzed using CyteMapper software and analysis tools. Cells are analyzed by a trained reviewer, and cell biomarker status is determined with a fluorescence intensity threshold.

RESULTS

CTCs were detected in 93% of patients (41/44) and in 86% of samples (67/78) collected, with the highest baseline average CTC counts in colorectal cancer. Changes in CTCs correlated with treatment response and tissue biomarker expression, e.g., HER2. Rapid decline or clearance occurred within days of effective systemic therapy initiation. Average CTC testing turnaround time was 5-7 days.

Gastrointestinal cancer subtype	# of patients	Initial Baseline Draw			
		Average # of CTCs per 7.5mL blood	Median # of CTCs per 7.5mL blood	Minimum # of CTCs per 7.5mL blood	Maximum # of CTCs per 7.5mL blood
Colorectal carcinoma	30	72	3	0	1378
Pancreatic cancer	4	2	2	0	6
Cholangiocarcinoma	3	8	3	1	23
Hepatocellular carcinoma	2	4	5	0	10
Neuroendocrine	2	9	9	2	18
Gallbladder cancer	1	6	6	3	8
Appendix cancer	1	2	2	1	3
Anal cancer	1	0	0	0	0

Table 1. Number of patient samples collected and tested of each Gastrointestinal (GI) cancer subtype with average, median, minimum, and maximum numbers of CTCs per 7.5mL blood in the initial baseline sample.

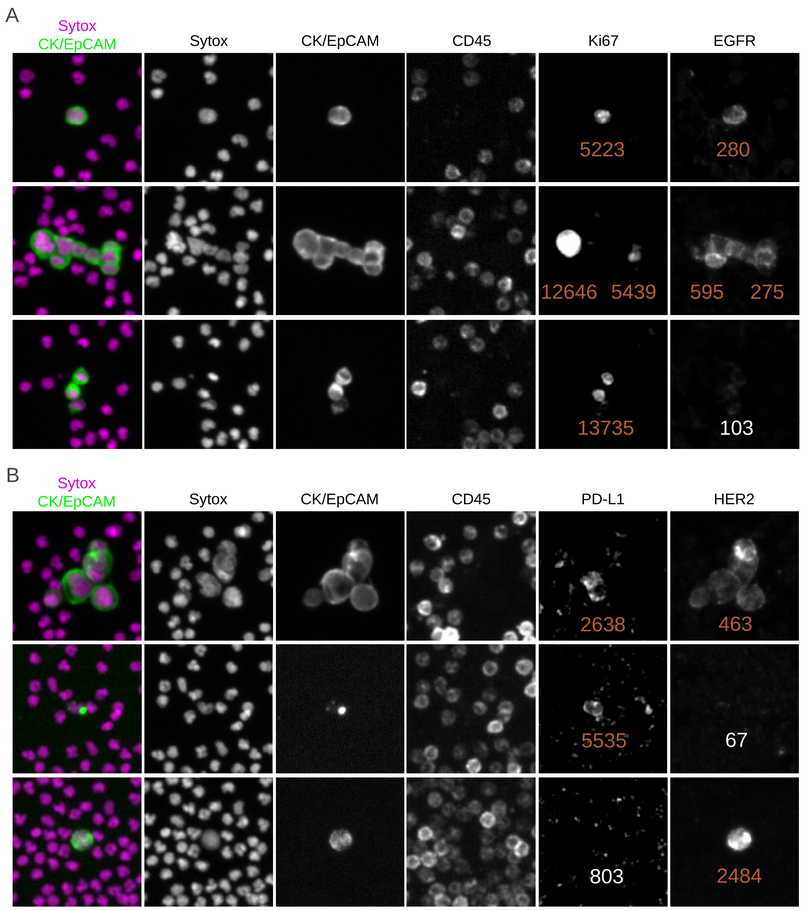

Figure 2. Representative images of dual biomarker staining on GI cancer patient samples. Representative CTC images from patient samples stained with Ki67/EGFR (A) or PD-L1/HER2 (B) biomarker assays. Biomarker Mean Fluorescent Intensity (MFI) shown for each CTC in copper (positive) or white (negative)

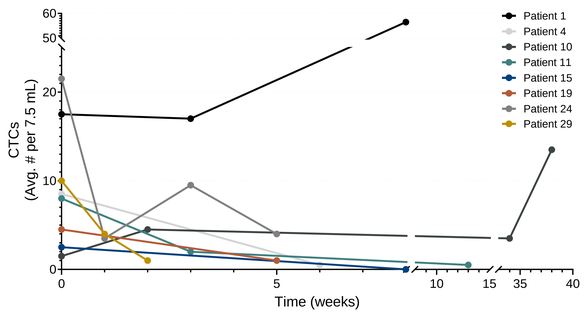

Figure 3. Real-time monitoring of CTC kinetics. Total number of CTCs found in blood samples taken over time from seven representative patients.

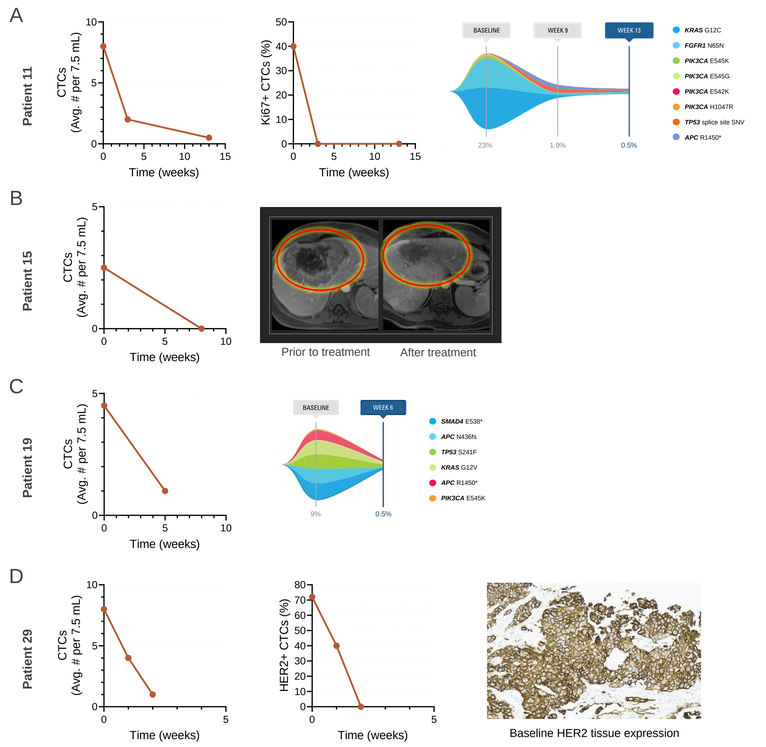

Figure 4. Patient response kinetics. (A) Real-time monitoring of Patient 11 CTC number and Ki67 biomarker positivity indicates early response to treatment prior to cfDNA mutational burden analysis. (B) Decrease in CTC number in Patient 15 indicate response to therapy confirmed via tumor imaging. (C) Results from real-time monitoring of CTC number, and cfDNA analysis correlate, indicating Patient 19 response to therapy. (D) Assessment of CTC number and HER2 biomarker positivity indicate Patient 29's early response to HER2 therapy. HER2 overexpression was detected via cfDNA, confirmed on IHC of tissue and on CTCs. After initiation of HER2 therapy, CTC numbers and HER2 expression showed a significant decrease.

Mutations found via cfDNA Sequencing Analysis	Mutations found via CTC Sequencing Analysis
SMAD4	IDH2 A177G
APC	NF1
TP53	ATM
KRAS G12V	KRAS G12V
PIK3CA	

Table 2. Sequencing Analysis. Genetic mutations with highest variant frequencies (VF%) found in patient 19 through cfDNA and single CTC sequencing. Mutations found in both cfDNA and CTCs indicated in copper.

CONCLUSIONS

- The RareCyte platform allows for quantitation and monitoring of clinically relevant protein biomarkers due to the high sensitivity, accuracy and specificity of developed assays
- CTC testing provides non-invasive, real-time monitoring, and early response assessment in cancer patients
- CTC results recapitulate patient responses detected via imaging scans and ctDNA analysis
- Mutation discrepancies between cfDNA and CTCs indicate biological differences between CTC DNA and plasma cfDNA, impacting patient therapeutic decisions