Novel method for the detection and evaluation of disease biomarkers on white blood cells from liquid biopsies

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Abstract #2292

BACKGROUND

Blood-based liquid biopsies of circulating tumor cells (CTCs) are a non-invasive tool with proven effectiveness in the prognosis, treatment selection, and stratification of cancer patients. In this study we demonstrate a novel application of the RareCyte platform, which was developed to find and analyze rare CTCs from blood samples, in evaluating white blood cell (WBC) sub-populations for disease biomarkers.

METHODS

Using AccuCyte®, an unbiased density-based method for collecting nucleated cells from whole blood, we transferred the nucleated cells to slides and stained for CD14, CD66b, CK/EpCAM, and Sytox to identify monocytes (CD14+/CD66b-), granulocytes (CD14-/CD66b+), and CTCs (CK+/EpCAM+/CD66b-/CD14-) as well as a drug-target biomarker to evaluate its expression across all cells. Slides were then imaged with CyteFinder®, an automated, multiparameter immunofluorescent (IF) microscopy system that applies machine learning algorithms for cell identification. Each slide contains millions of cells, so we developed novel scanning and analysis algorithms to quantify WBC sub-populations in addition to rare CTCs.

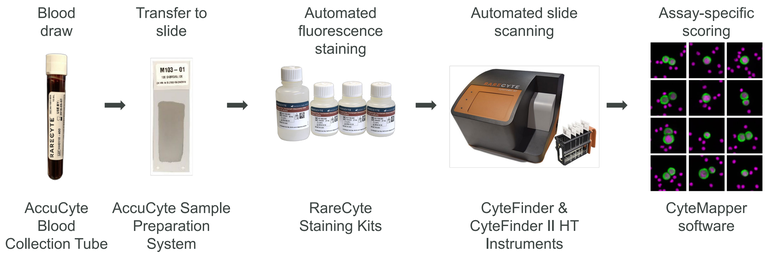

Figure 1. RareCyte platform workflow. Blood is collected into AccuCyte Blood Collection Tubes (BCTs). Nucleated blood cells are processed to slides using the AccuCyte Sample Preparation System. Slides are stained with assay-specific RareCyte staining kits using the Ventana® DISCOVERY® ULTRA automated slide staining system. 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

The RareCyte platform was able to detect individual white blood cells and quantify monocyte and granulocyte subpopulations. Our results correlated strongly with standard CBC (Complete Blood Count) readings from multiple donors. Additionally, the monocyte and granulocyte populations were evaluated for expression of a drug-target biomarker. While expression of the biomarker was observed on both granulocytes and monocytes, monocytes had a significantly higher expression, a critical finding for future implementation of this drug.

Furthermore, we utilized the RareCyte platform to detect and evaluate white blood cell subpopulations in combination with spiked CTCs. Clusters of white blood cells with CTCs are known to portend a worse diagnosis for patients. Our system offers a novel method of characterizing and evaluating WBCs in combination with CTCs as well as quantifying expression of biomarkers across multiple rare and non-rare cell populations.

	% Monocytes		% Granulocytes	
	CBC	RareCyte Assay	CBC	RareCyte Assay
Donor 1	8.3%	8.4%	65.6%	63.5%
Donor 2	7.4%	6.4%	73.3%	72.0%
Donor 3	7.1%	6.7%	57.7%	51.3%

Table 1. Detection of monocytes and granulocytes. Whole blood was collected from each donor in EDTA and AccuCyte BCTs. CBCs were measured from blood samples collected in EDTA tubes. Whole blood collected in AccuCyte BCTs was processed, and the isolated buffy coat was stained for CD14, CD66b, the biomarker and Sytox. Cells defined as monocytes (CD14+/CD66b-) or granulocytes (CD66b-/CD14+) were detected using CyteFinder and quantified compared to the total number of cells. The percent of monocytes and granulocytes detected using the RareCyte assay was averaged from at least two slides for each sample.

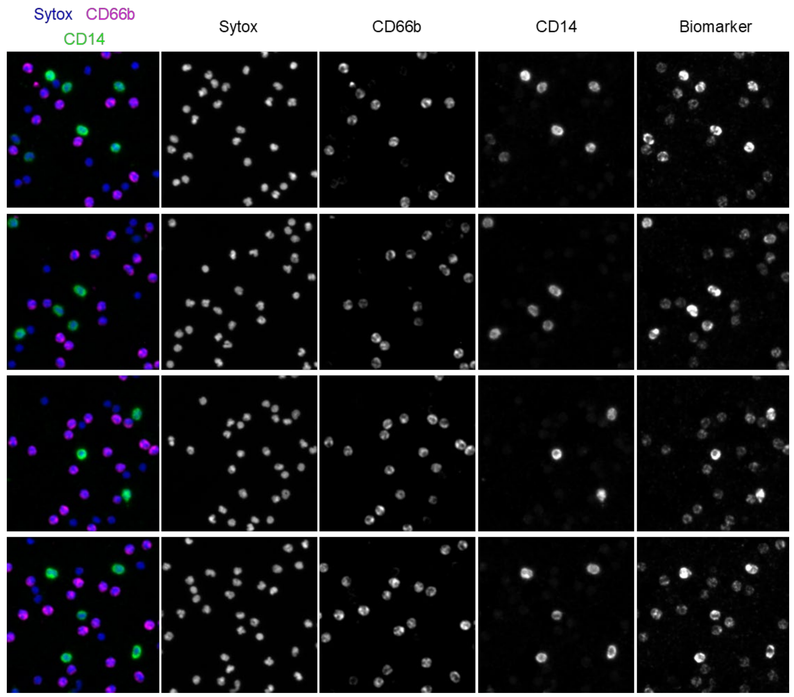

Figure 2. Representative images of biomarker expression across monocytes and granulocytes. Whole blood was processed using AccuCyte, and the isolated buffy coat was stained for CD14, CD66b, Sytox, and a Biomarker. Each row provides staining results for a defined area around a detected monocyte. Four representative areas were selected for illustration.

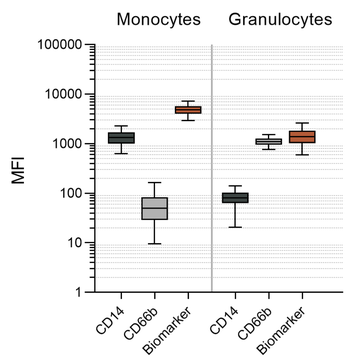

Figure 3. Mean Fluorescence Intensity (MFI) distribution of each marker on monocytes and granulocytes. The MFI distribution of CD14 (dark grey), CD66b (light grey), and the biomarker (copper) on monocytes and granulocytes. Each box represents the aggregate data from three replicate slides. Whiskers span 5 – 95 percentile.

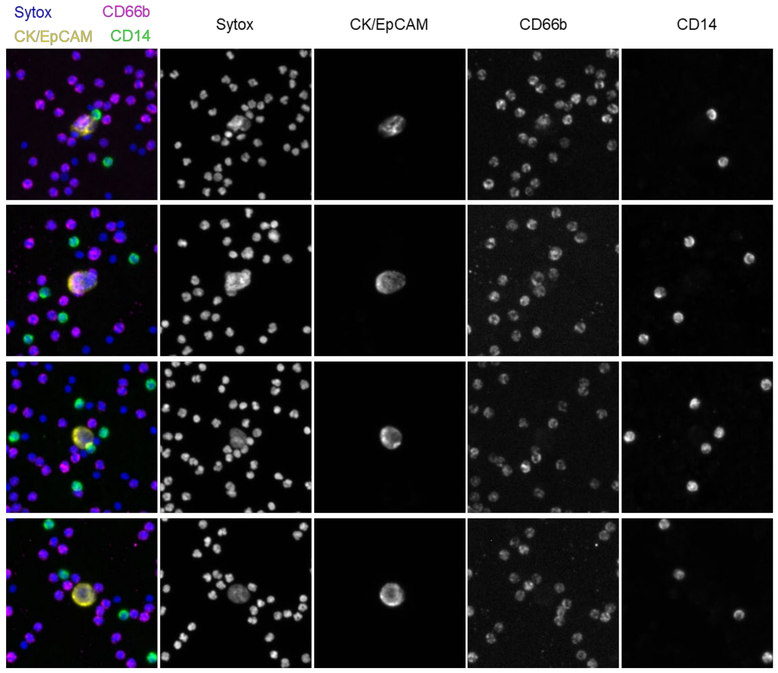

Figure 4. Representative images of spiked CTCs and white blood cells. BT474 breast cancer-derived cells were spiked into whole blood, which was processed using AccuCyte, and the isolated buffy coat was stained for pan-Cytokeratin (CK), EpCAM, CD14, CD66b, Sytox, and the biomarker. Each row provides staining results for a defined area around a single spiked CTC. Four representative CTCs were selected for illustration.

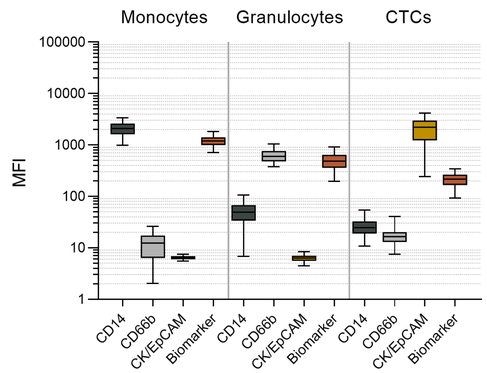

Figure 5. MFI distribution of each marker on monocytes, granulocytes, and spiked CTCs. The MFI distribution of CD14 (dark grey), CD66b (light grey),CK/EpCAM (gold), and the biomarker (copper) on monocytes, granulocytes and spiked CTCs. Each box represents the aggregate data from two replicate slides. Whiskers span 5 – 95 percentile.

CONCLUSIONS

- A novel method to identify and evaluate expression of biomarkers across multiple rare and non-rare cell populations was developed.
- Method incorporates the benefits of high-throughput data analysis and biomarker quantitation with the specificity and sensitivity of rare cell detection.
- High-resolution multiplexed immunofluorescent imaging provides cell morphology, biomarker quantitation, and biomarker localization information.

❖ RareCyte platform presents an exciting opportunity for a deeper evaluation of patients and their prognosis through liquid biopsies.