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Multi-analyte subtyping of circulating cancer derived cells for screening Immunotherapeutic targets

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ABSTRACT

In tissue biopsies, cancer samples are micro-sectioned, producing multiple semi-identical specimens that are analyzed and subtyped proteomically and genomically with numerous biomarkers. A common issue with biomarker panels of tissue biopsies is that over time and after intervention, the evolution of the tumor can produce distinctly different cell subpopulations, with proteomic and genomic patterns inconsistent with the original biopsy. In blood based biopsies (BBBs), multiple blood samples can be taken sequentially, but clinical utility is typically limited to cell enumeration, as only 2-3 biomarkers can be used. Here, we describe a technique that provides the ability to sequentially restain isolated rare cells from BBBs with numerous additional subtyping biomarker panels for use in screening therapeutic targets.

INTRODUCTION

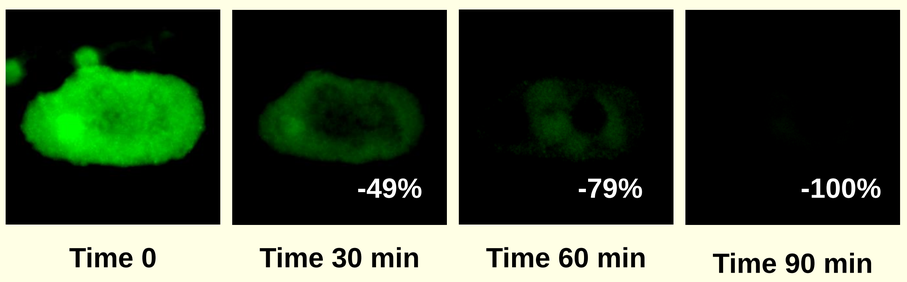
Circulating Tumor Cells (CTCs) are an indicator of malignant disease, used to monitor therapy response and predict outcomes in late stage patients.[1-4] However, CTCs are not common in all diseases, and the low frequency makes the tracking of therapeutic response difficult.

Cancer Associated Macrophage-like cells (CAMLs) are a newly-defined circulating immune cell type, described as a subtype of circulating stromal cells. They express actionable drug targets (e.g. TIE-2, CXCR4, PD-L1, etc), have been shown to be present in all stages of cancer, are responsive to cancer treatments, and are found in multiple cancer types.

EMT-like cells are found in almost all patients with solid malignancies and are theorized as the aggressive CTC subtype that initiates tumor metastases. Typically, EMT-like cells are identified by their down regulation of EpCAM, and/or Cytokeratin, and up regulation of Vimentin, and/or N-Cadherin. [1-4]

We have reported that CellSieve™ microfilters rapidly and efficiently isolate the three most common circulating cancer associated cells (CAMLs, CTCs, and EMTs) from whole peripheral blood, making it possible to study all cell types in conjunction with, and in relation to, therapy response in a variety of malignant diseases. [3-4]

Figure 1. Decrease of signal over time.



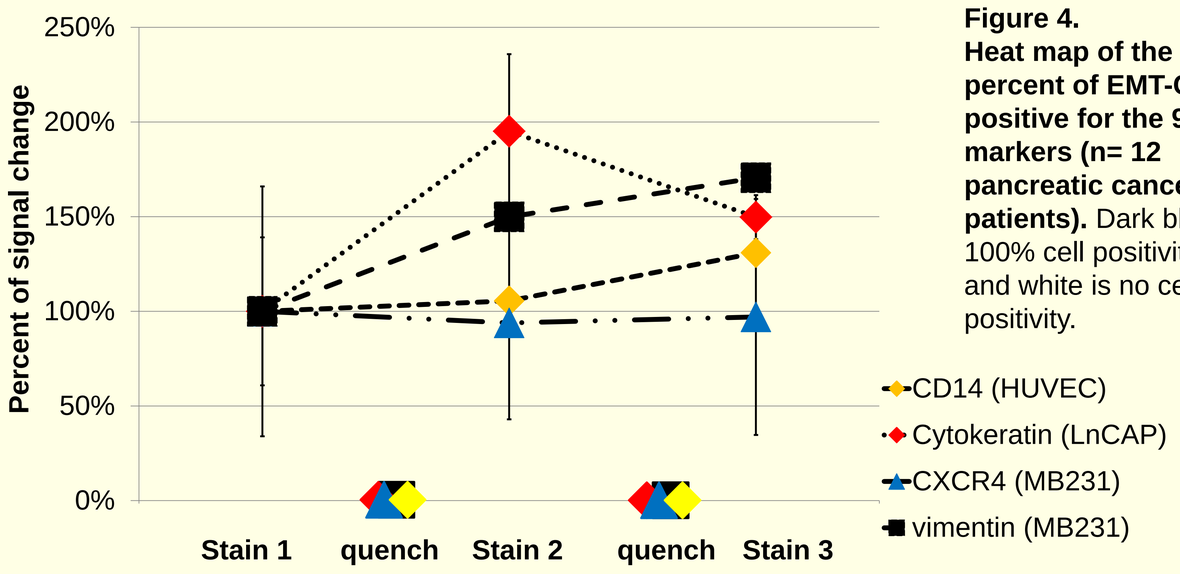


Figure 2. Representative examples of the percent change of signal during multiple rounds of the QUAS-R technique

MATERIALS & METHODS

CellSieve™ microfilters were used to isolate CTCs, CAMLs, and EMTs cells from 7.5 mL of peripheral blood from patients with breast, lung or pancreatic cancer. Collected cells were fixed, permeabilized, and stained with DAPI and antibodies against cytokeratin 8, 18 and 19, EpCAM and CD45. Cancer derived cells were identified and imaged under a fluorescent microscope. After cell identification, enumeration and subtyping, cells on the filter were marked and samples archived. Archived samples can be reanalyzed for additional markers. The stain is removed using the QUAS-R technique, i.e., each cell is quenched of fluorescence) and restained for additional markers of interest. Each cell can be sequentially stained, quenched and reanalyzed for 2 additional sets of bio-relevant markers. The same cells are reevaluated, allowing IHC based scoring of each cell for multiple markers.

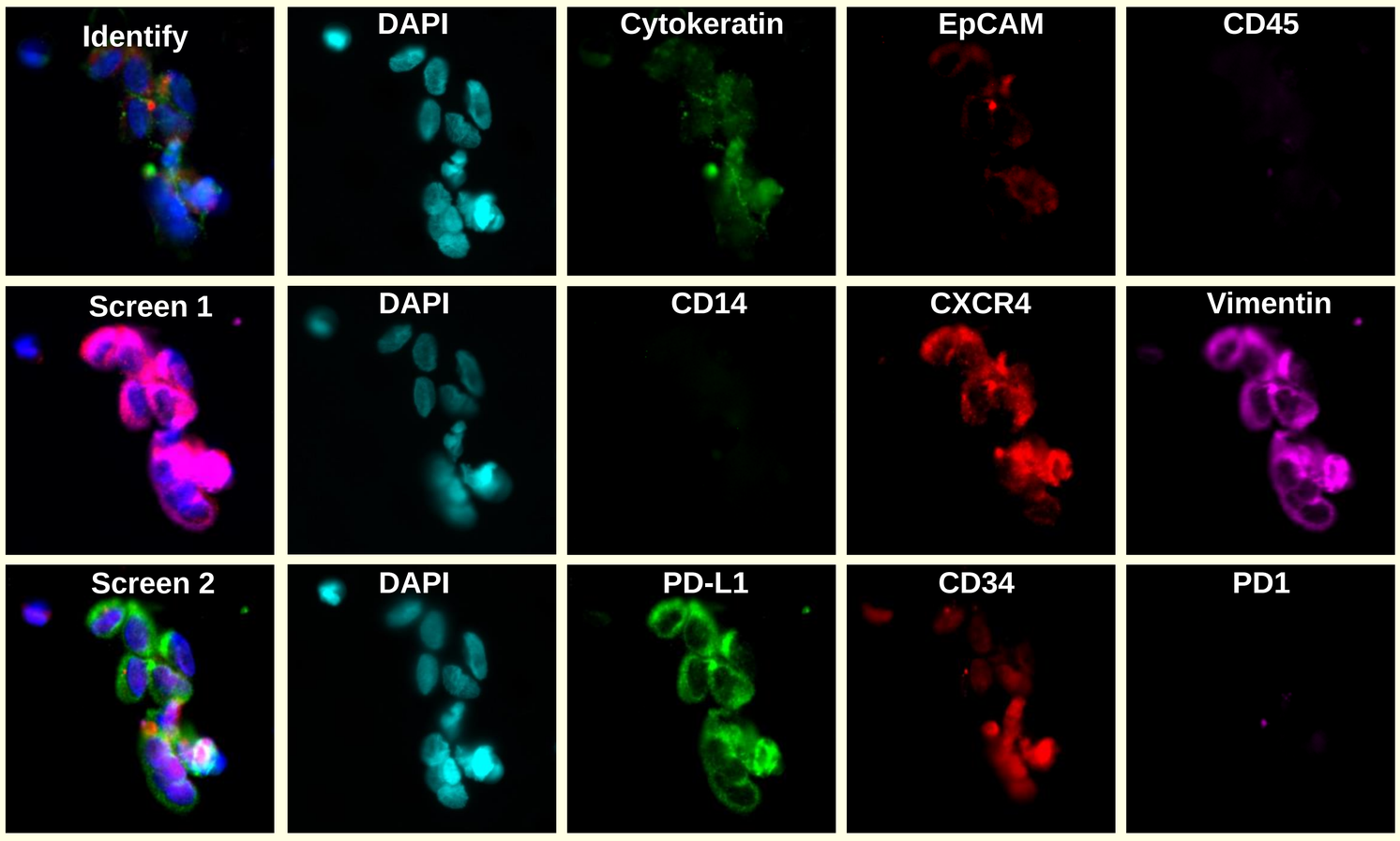
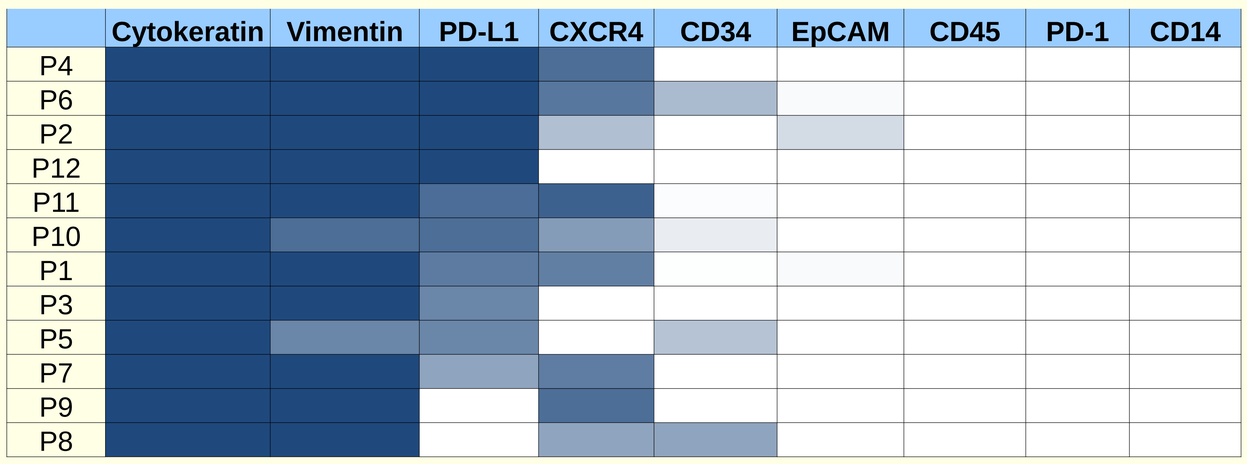


Figure 3. Sequential staining, qualification and quantification with an array of biomarker panels against the same cell cluster. An EMT-like CTC cluster, identified with a "classical" CTC stain (top), a panel of motility markers (middle), and a panel of immune activation markers (bottom).

RESULTS

- At least one cancer associated cell (i.e. CTC, EMT or CAML) was identified in 95% of samples tested.
- Cells could be identified, marked, fluorescence removed, and sequential staining performed.
- No degradation was observed in cell surface/intracellular markers for 3 rounds of QUAS-R restaining.
- We performed sequential staining of cancer derived cells, quantifying the expression patterns of numerous applicable drug targets (e.g. EpCAM, CD31, CD34, CXCR4, Vimentin, PD1, and PD-L1).

Figure 4. Heat map of the percent of EMT-CTCs positive for the 9 markers (n= 12 pancreatic cancer patients). Dark blue is 100% cell positivity and white is no cell positivity.



CONCLUSIONS

- Our data demonstrates that one can sequentially screen, analyze and track drug applicable targets using a panel of cancer associated cells from blood based biopsy.
- Using multiple cell types provides a greater amount and broader variety of information than single cell analysis.
- This approach can be used in research, patient selection and companion diagnostics, and/or monitoring of response for immunotherapy.

References

1. Adams DL, et al "Circulating giant macrophages as a potential biomarker of solid tumors." Proc Natl Acad Sci, 111(9):3514-3519, 2014
2. Adams DL, et al "Precision microfilters as an all in one system for multiplex analysis of circulating tumor cells" RSC Advances, 6(8):6405-6414, 2016
3. Plaks, V., et al., "Circulating Tumor Cells." Science 341(6151): 1186-1188, 2013.
4. Adams DL, et al, "Cytometric Characterization of Circulating Tumor Cells Captured by Microfiltration and Their Correlation to the CellSearch® CTC Test." Cytometry A 87(2): 137-144, 2015.

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