



# A Cell Based Liquid Biopsy for Immunotherapeutic Drug Development

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## ABSTRACT

Biomarker analysis is used in a variety of steps in drug development and as a surrogate to therapy response in patients. The sample source typically used to analyze drug companion biomarkers is a tissue biopsy. Though the procedure varies, in general biopsies are invasive, costly, and can be dangerous; and as such, they are seldom repeated after initial diagnosis. Exacerbating these issues 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. Here we suggest an alternative source of tumor biomarkers, circulating cancer associated cells isolated, from the blood of cancer patients.

In the context of drug development, there are numerous advantages to using patient blood as a source for the biomarkers. Cells from the blood are plentiful, can be used to evaluate the expression of drug targets in real time, and they can be obtained repeatedly. This is especially important as the tendency of cancers to evolve over time, and in response to treatment, requires oncologists to continuously re-evaluate the tumor biology and therapy. While a noninvasive sequential testing method is highly desirable, to date circulating tumor cells (CTCs) alone have not met the numerical threshold to be used for all patients, and with implementation of stromal targetable therapies, one must now look beyond CTC analysis.

## INTRODUCTION

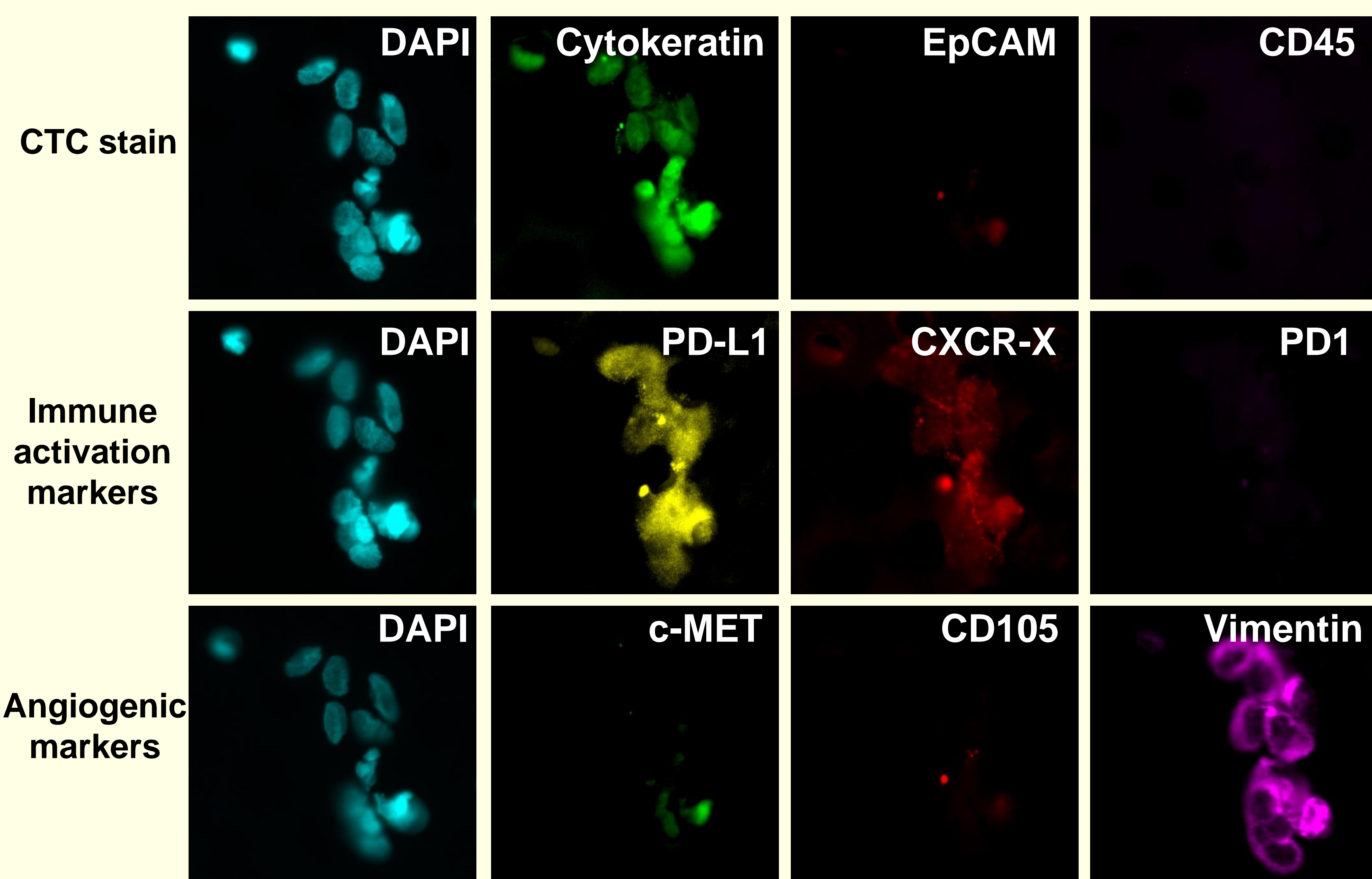
The four most common cancer associated cells found in the blood of cancer patients are CTCs, circulating cancer associated macrophage-like cells (CAMLs), epithelial-mesenchymal transition-like CTCs (EMT-like cells) and circulating endothelial cells (CECs).

CellSieve™ microfilters are lithographically fabricated membranes with high porosity and precise pore dimensions<sup>3-4</sup>. We have reported that CellSieve™ rapidly and efficiently isolate CAMLs, CTCs, EMTs and CECs from whole peripheral blood, making it possible to study all of these cell types in conjunction with, and in relation to, therapy response in a variety of solid tumors.<sup>3-4</sup>

CTCs are an indicator of malignant disease, used to monitor therapy response and predict outcomes in late stage patients.<sup>1-4</sup> However, CTCs are not common in all diseases, and the low frequency makes tracking therapeutic response difficult.

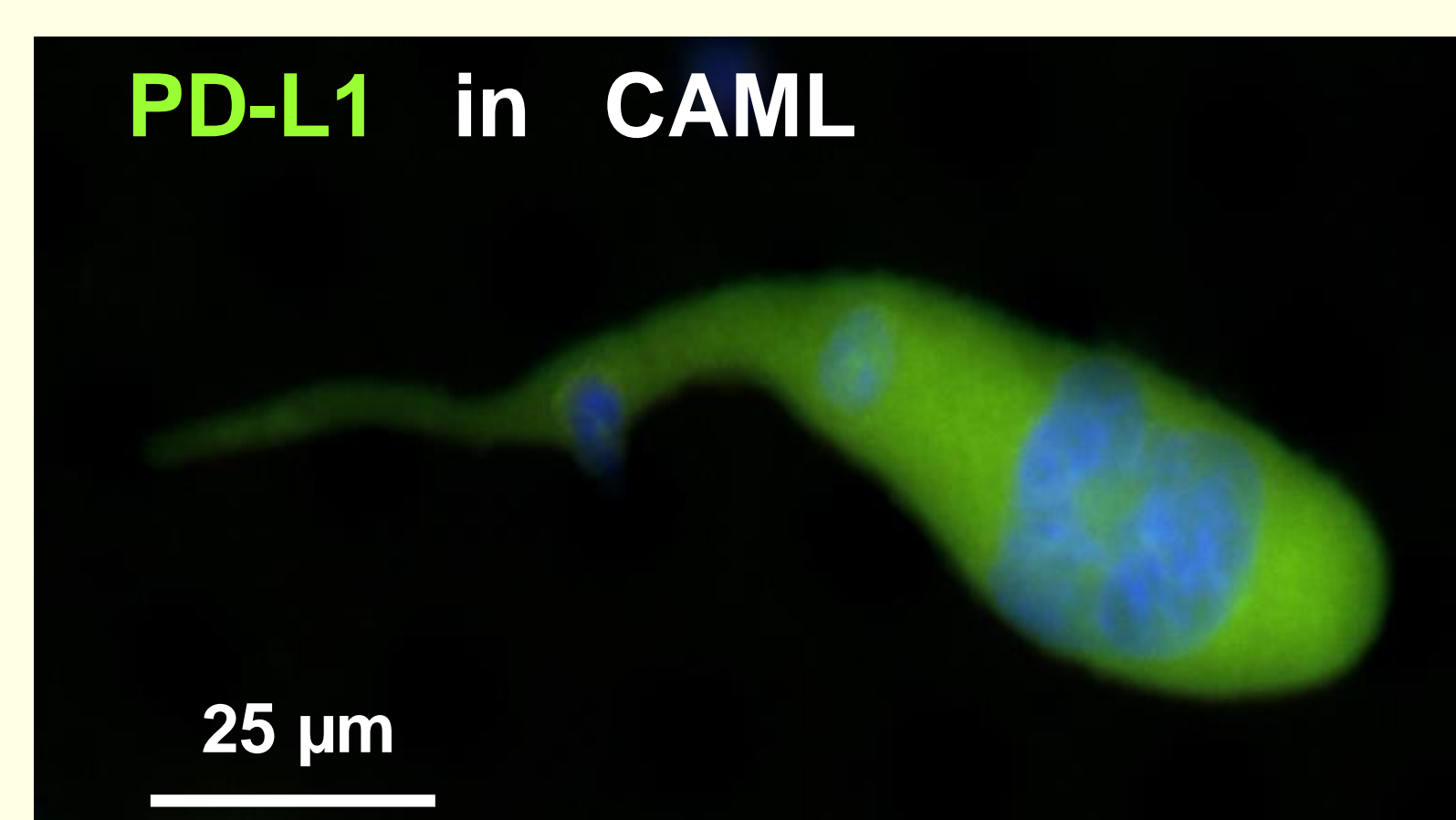
CAMLs are a newly-defined circulating immune cell type, a subtype of circulating stromal cells, known to express actionable drug targets (e.g. PD-L1, CXCR4, etc)<sup>1</sup>. Present in all stages of cancer, CAMLs are responsive to cancer treatments, and are found in the blood of most solid tumor patients.<sup>1</sup>

EMT-like cells are also found in almost all patients with solid malignancies and have been theorized as the aggressive CTC subtype that initiates metastasis. Typically, EMT-like cells are identified by their down regulation of EpCAM, and/or Cytokeratin, and up regulation of Vimentin, and/or N-Cadherin.<sup>1-4</sup>

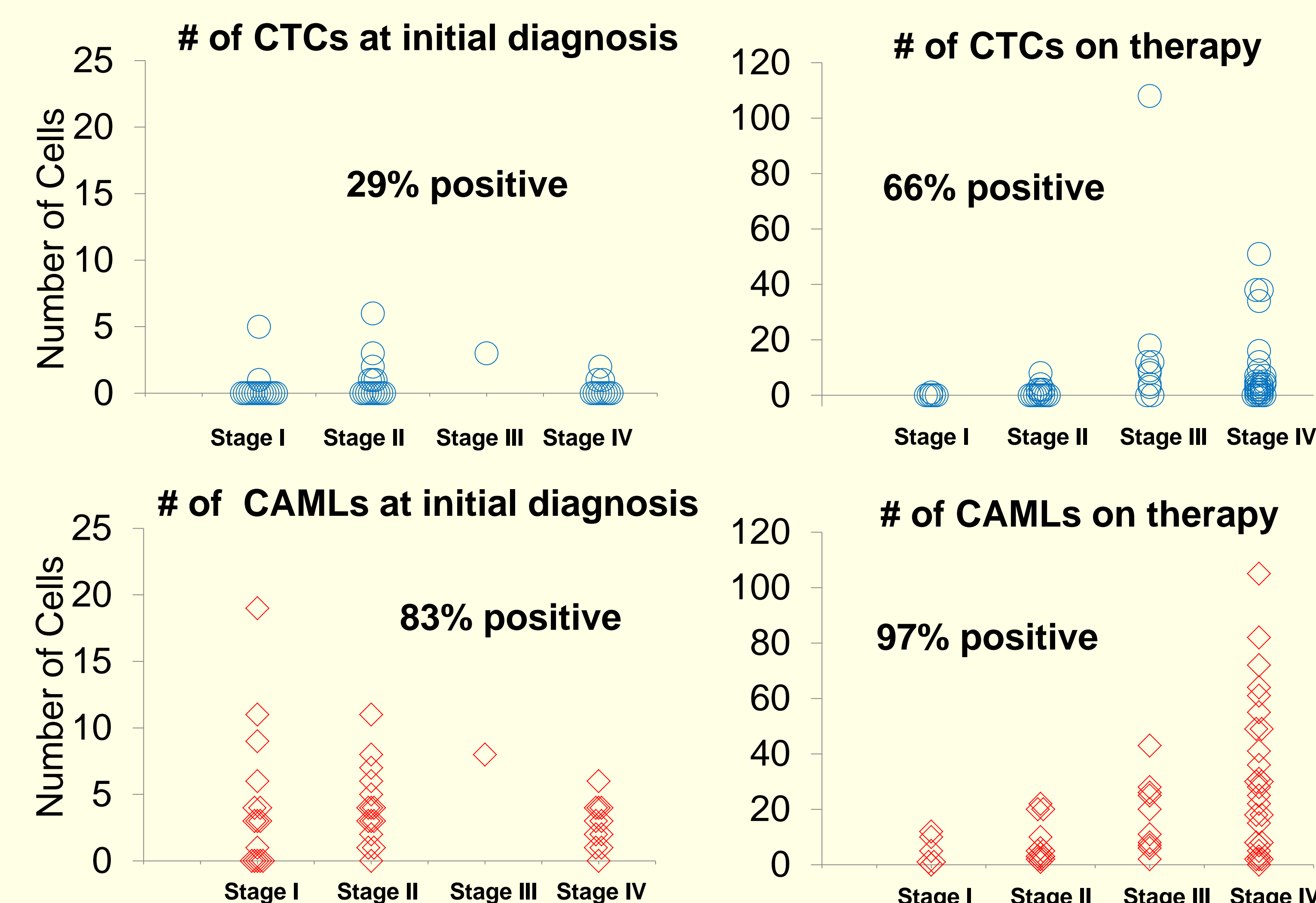


**Figure 1. Sequential staining, qualification and quantification with an array of biomarker panels against the same cell cluster. An EMT-like CTC cluster, identified with a “classical” CTC stain (top), a panel of immune activation markers (middle), and with a panel of angiogenic markers (bottom).**

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**Figure 2. A 125 μm long CAML expressing PDL1 (green) with DAPI (blue). PDL1 expression may result by the engulfment of CTCs and tumor debris.**



**Figure 3. Positivity in CAMLs and CTCs in Cancer Patients and their response to therapy. While CTCs have lower rate of positivity in cancer patient samples, they are prognostically valuable and decrease in response to therapy. CAMLs are not prognostic, but have high rate of positivity and are highly responsive to therapy.**

## MATERIALS & METHODS

CellSieve™ microfilters were used to isolate CTCs, CAMLs, EMTs and CECs from 7.5 mL of whole peripheral blood based on size. CellSieve™ filters have uniform 7 micron pores, with 160,000 pores distributed uniformly over a 9 mm diameter area. Collected cells are fixed, permeabilized, and stained with DAPI and antibodies against cytokeratin 8, 18 and 19, EpCAM and CD45. CD45 is used to exclude white blood cells. After cell identification, enumeration and subtyping; via imaging cells under a fluorescent microscope; cells on the filter are marked and the sample archived. When desired, archived samples are retrieved from storage, and cells quenched and restained for additional markers of interest. Typically each cell can be sequentially stained, quenched and reanalyzed for 12 bio-relevant markers. In each case, the same cells are reevaluated, allowing for IHC based scoring of each individual cell with a multiplex biomarker analysis.

## RESULTS

- Some, or all, of the four types of cancer associated cells (CTCs, CAMLs, EMTs and CECs) can be found in blood samples of cancer patients with solid tumors.
- CAMLs have the highest rate of positivity, found in all stages of most cancers (>90% sensitivity).
- EMT-like CTCs are the found in highest numbers (median=20), but less frequent than CAMLs (~60% sensitivity).
- Cancer associated cells isolated from patient blood can be sequentially stained, qualified and quantified with an array of biomarkers (e.g. TIE-2, PSMA, HER-2, c-MET, CD105, PD1, PD-L1).
- We have processed sequential staining for many cancers (e.g. pancreatic, breast, kidney and lung).

## CONCLUSIONS

- Our data suggests that circulating cancer associated cells can be sequentially analyzed in order to track multiple drug targets from a single sample.
- With overall frequency of 90% and median frequency of 27 cells/sample, circulating cancer associated cells in blood provides a greater amount, and broader variety, of information than CTC analysis alone.
- This work has implications in research, patient selection in trials, companion diagnostics & monitoring of response of immunotherapy.

## References

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