

# Cancer associated macrophage-like cells in the early detection of solid tumors

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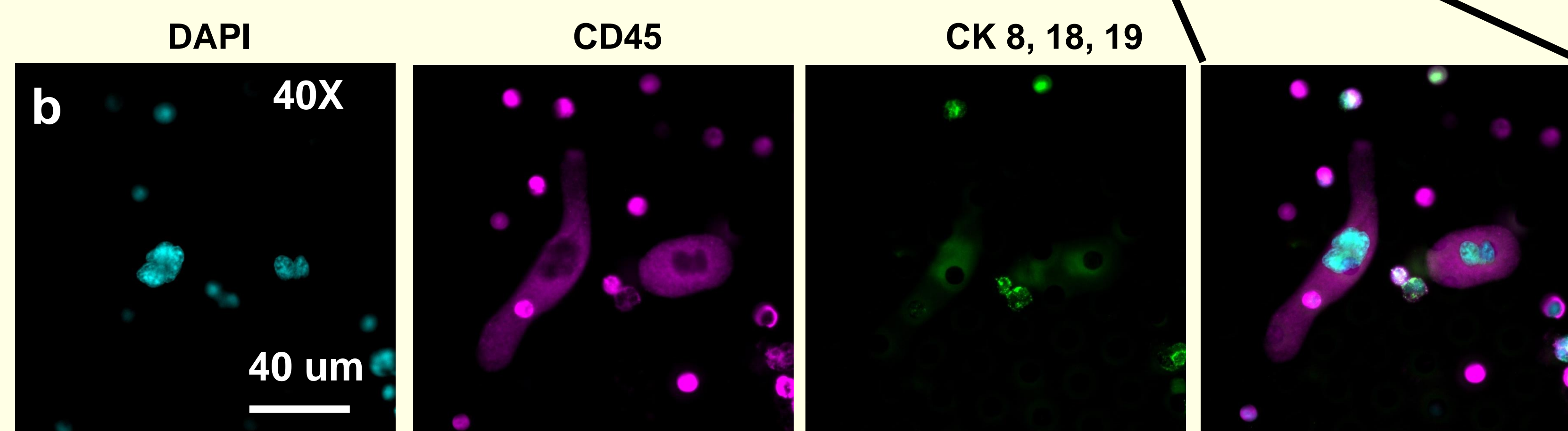
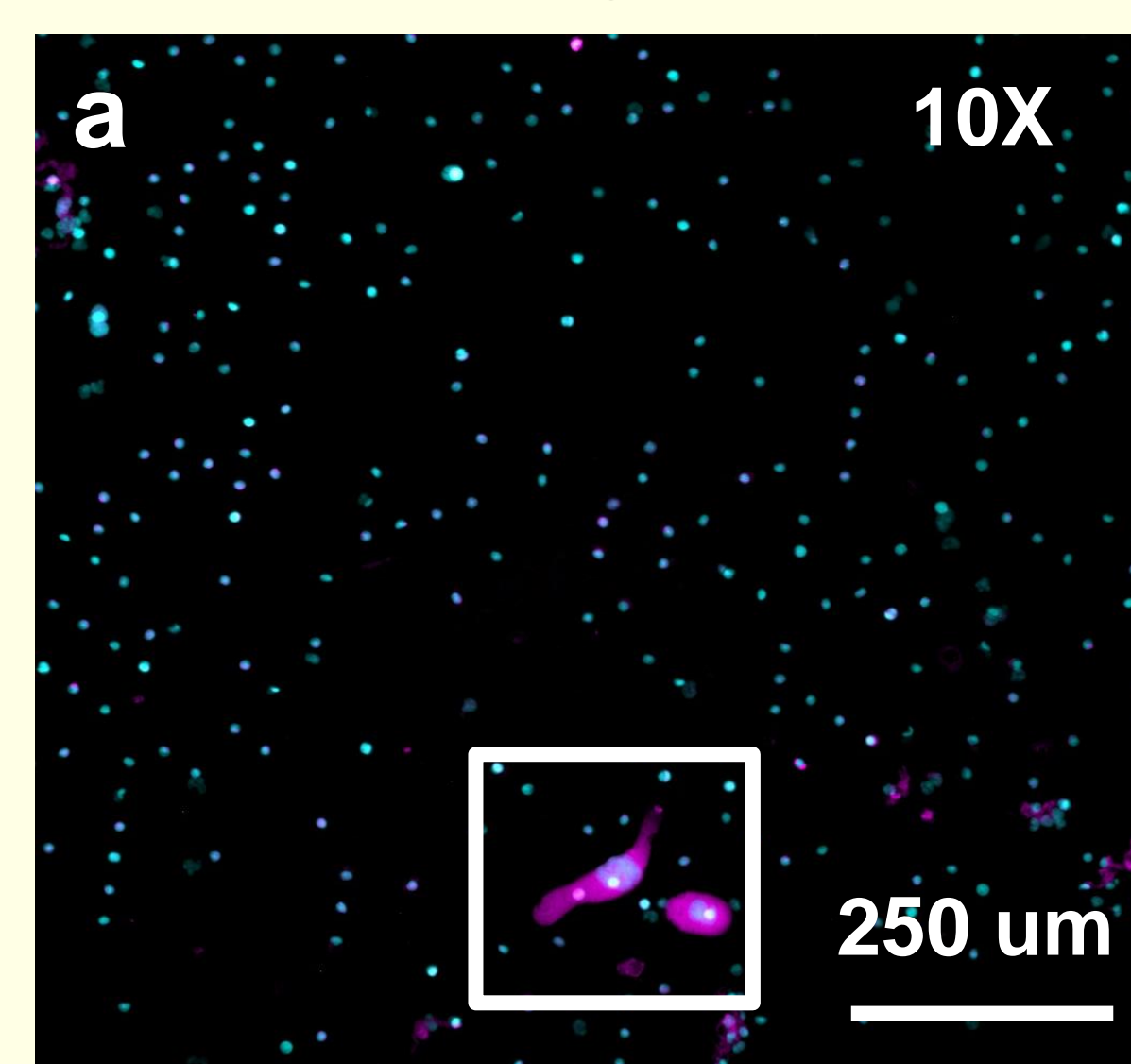
## ABSTRACT

Blood-based biopsies can be used as a non-invasive method to recover a variety of cancer associated circulating cells, including Circulating Tumor Cells (CTCs) and Circulating Cancer Associated Macrophage-like cells (CAMLs) from the blood of cancer patients. CAMLs are a newly-defined circulating immune cell type, described as a subtype of circulating stromal cells and found specifically in patients with malignant disease. We studied the peripheral blood of cancer patients to ascertain the prevalence, specificity and sensitivity of CAMLs in relation to their disease status at presentation. We compared a variety of benign and malignant diseases, along with matched healthy control blood samples. We supply evidence that this previously unidentified circulating cell can be used as a screening tool to detect solid tumors in numerous malignancy subtypes in all disease stages.

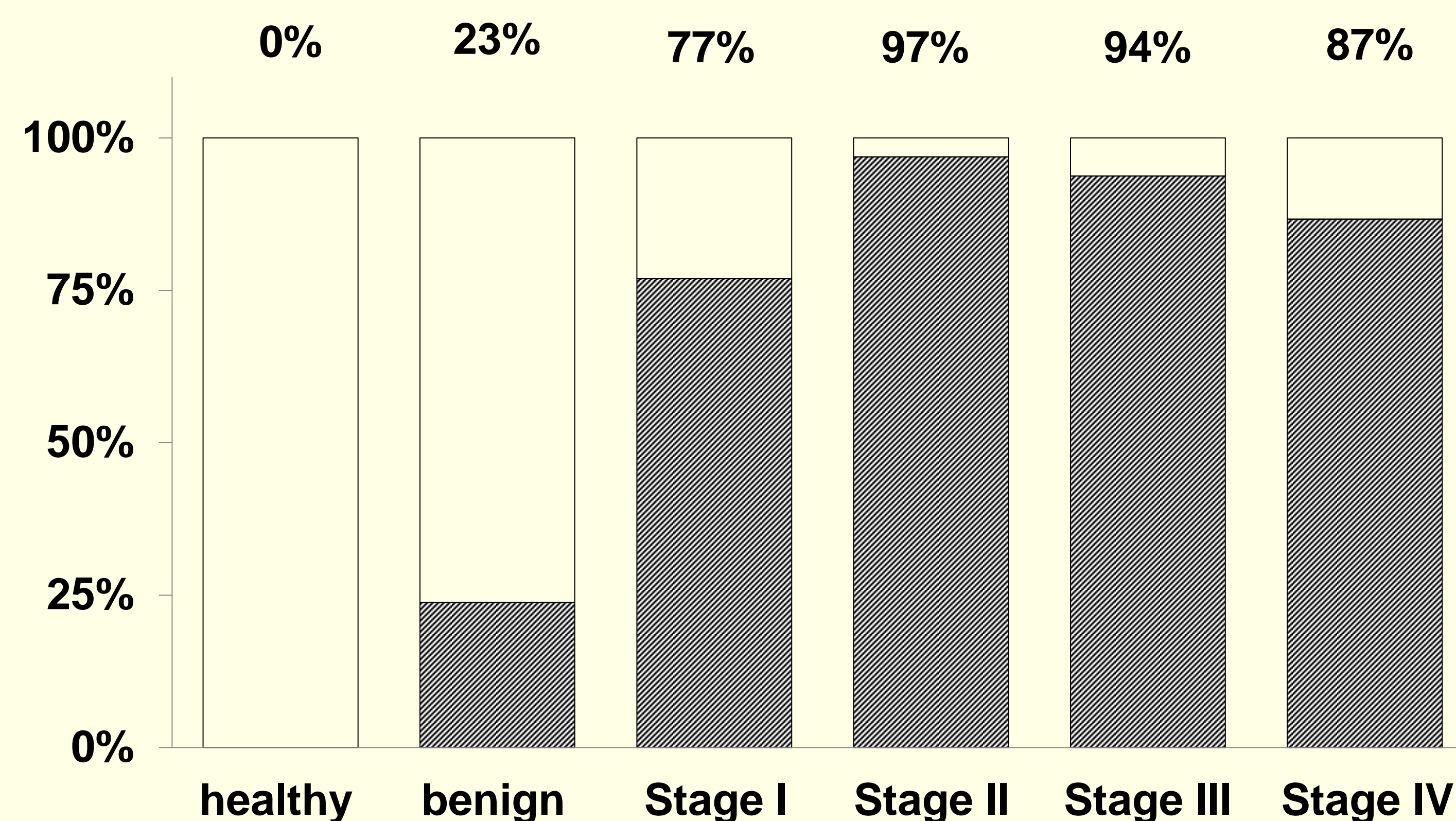
## INTRODUCTION

CAMLs are specialized myeloid cells transiting the circulation of patients in all stages of cancer. They are responsive to cancer treatment and are found in multiple cancer types<sup>1,2</sup>. However, though seen by numerous groups, these cells have remained largely unstudied, and their clinical and biological value in malignancies remains uninvestigated.

Size exclusion is a technique for isolating large cells from peripheral patient blood irrespective of their surface marker expression. CellSieve™ microfilters are precision size exclusion membranes capable of rapidly and efficiently isolating both CAMLs and CTCs from whole blood, making it possible to study both cell types in conjunction with and in relation to malignant disease<sup>1-4</sup>.



**Figure 1. Isolation and identification of CAMLs by size and nuclear size**  
(a) CAMLs are easily identified under 10X magnification from a prostate patient  
(b) Under 40X magnification the large polyploid nuclear structure can be seen (DAPI). These cells are most of the time positive for CD45 and weakly positive for cytokeratin



**Figure 2. Presence of CAMLs in relation to stage of malignant disease, in healthy controls, and in patients with benign masses.**

## References

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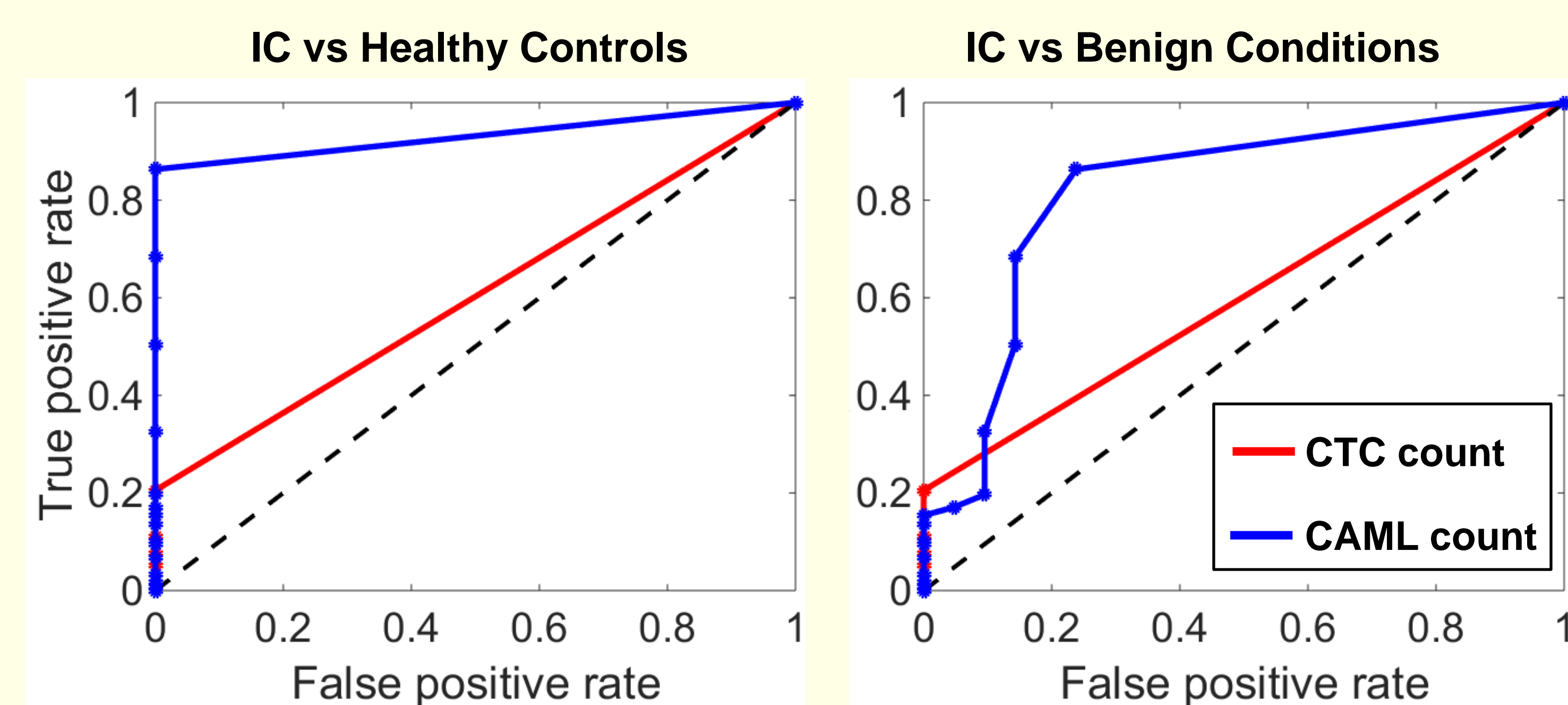
## MATERIALS & METHODS

Peripheral blood samples from cancer patients were provided by University of Maryland, Greenebaum Cancer Center, Northwestern University, The Medical College of Wisconsin, Fox Chase Cancer Center, and Duke University. We ran a prospective blinded study to isolate CAMLs from patients with known invasive carcinomas (n=117), healthy control samples (n=40) and patients with benign non-malignant conditions (n=21). The patient distribution included Stage I (n=39), Stage II (32), Stage III (16), Stage IV (30); breast (n=31), pancreatic (n=22), lung (n=38), and prostate (n=26) cancers. CellSieve™ microfilters were used to isolate CTCs and CAMLs from 7.5 mL of whole peripheral blood. The 7 μm pore size of CellSieve™ is capable of isolating both CTCs and CAMLs based on size. Collected cells were fixed, permeabilized, and stained with DAPI and antibodies against cytokeratin 8, 18 and 19, EpCAM, and CD45. CAMLs were defined as enlarged, multinuclear cells with diffuse cytoplasmic cytokeratin staining; and they can be CD45+ or CD45-. CTCs were defined as filamentous cytokeratin cells that are CD45-.

## RESULTS

- CAMLs were found in 87% patients with confirmed malignant disease.
  - CAMLs were found in 77% of stage I, 97% of stage II, 94% of Stage III, and 87% of Stage IV patients, regardless of cancer type.
  - CAMLs were found in 77% of prostate, 100% of pancreatic, 82% of lung, and 90% of breast patient samples.
- CAMLs were absent in 16 of 21 patients with benign masses.
- CTCs were found in 21% of the same patient cohort, averaging 0.9 cells per sample.
- Neither CAMLs nor CTCs were found in any healthy individuals (n=40).

Invasive Carcinomas (IC) vs Healthy Controls					
	Sensitivity (CI95%)	Specificity (CI95%)	AUC	PPV (CI95%)	NPV (CI95%)
CAMLs	87% (80-93%)	100% (91-100%)	0.93 (0.89-0.96)	100% (97-100%)	73% (59-84%)
CTCs	21% (14-29%)	100% (91-100%)	0.60 (0.57-0.64)	100% (86-100%)	30% (22-39%)
Invasive Carcinomas vs Benign conditions					
CAMLs	87% (80-90%)	76% (53-92%)	0.82 (0.67-0.89)	95% (89-99%)	52% (33-70%)
CTCs	21% (14-29%)	100% (84-100%)	0.60 (0.57-0.65)	100% (86-100%)	18% (12-27%)



**Figure 3. ROC curves using CAMLs (blue) or CTCs (red) for Invasive carcinomas vs healthy controls (left) or Invasive carcinomas vs Benign conditions (right)**

## CONCLUSIONS

- Highly differentiated monocytic cells transit the blood of cancer patients
- CAMLs can be used as a non-invasive blood based biopsy, to detect the anatomical presence of solid malignancies.
- CAMLs sensitivity and specificity suggests its role as a blood biomarker for early stage cancer screening in a broad population and at-risk groups.
- This data suggests that larger studies be done to determine the use of CAMLs as a screening tool for cancer.

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