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Contact:: cmtang@creatvmicrotech.com

Cancer associated macrophage-like cells as a blood-based biomarker for the screening of solid tumors

Daniel L Adams[1], R. Katherine Alpaugh[2], Massimo Cristofanilli[3], Stuart Martin[4], Saranya Chumsri[5], Raymond C. Bergan[6], Susan Tsai[7], Martin Edelman[4], Peixuan Zhu[8], Shuhong Li[8], Olga V. Makarova[9], Platte Amstutz[10], Cha-Mei Tang[10], Jeffrey R Marks[11]

[1] Creatv MicroTech, Inc., Monmouth, NJ 08852, [2]Fox Chase Cancer Center, Philadelphia, PA 19111, [3]Thomas Jefferson University Hospital, Philadelphia, PA 19111, [4]University of Maryland School of Medicine, Baltimore, MD 21201, [5]Mayo Clinic Cancer Center, Jacksonville, FL 32224, [6]Oregon Health and Science University, Portland, OR 97239, [7]Medical College of Wisconsin, Milwaukee, WI 53226, [8]Creatv MicroTech, Inc. Rockville, MD 20850, [9]Creatv MicroTech, Inc., Chicago, IL 60612, [10]Creatv MicroTech, Inc., Potomac, MD 20854, [11]Duke University, Durham, NC 27710

ABSTRACT

Blood-based testing can be used as a non-invasive method to recover and analyze Circulating Tumor Cells (CTCs), and Circulating Cancer Associated Macrophage-Like cells (CAMLs), from the blood of cancer patients for numerous clinical implications. However, while the clinical utility of CTCs is a well studied field, CAMLs are a largely unstudied event. Here, we studied the peripheral blood of cancer patients to ascertain the prevalence, specificity and sensitivity of CAMLs in relation to disease status at presentation in benign and malignant diseases. We supply evidence that this previously unidentified circulating immune cell may be used as a screening tool to detect solid tumors in various malignancies, irrespective of disease stage.

INTRODUCTION

CAMLs are specialized myeloid cells that are found transiting the circulation of patients in all stages of cancer. They are responsive to cancer treatment and are found in multiple cancer types.[1,4] 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 and is ideal for isolating CAMLs and CTCs[1-4]. CellSieve™ microfilters are 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. [3-4]

CellSieve™ microfilters are used to isolate CAMLs and CTCs from 7.5 mL of whole peripheral blood. Cells are fixed, permeabilized, and stained with DAPI, antibodies against cytokeratin 8, 18 and 19, CD14, and CD45. CAMLs are described as enlarged, multinuclear cells with diffuse cytoplasmic cytokeratin staining, and/or CD14+, and/or CD45+.[1] CTCs are defined as filamentous cytokeratin containing cells that are CD45 negative.[1,4]

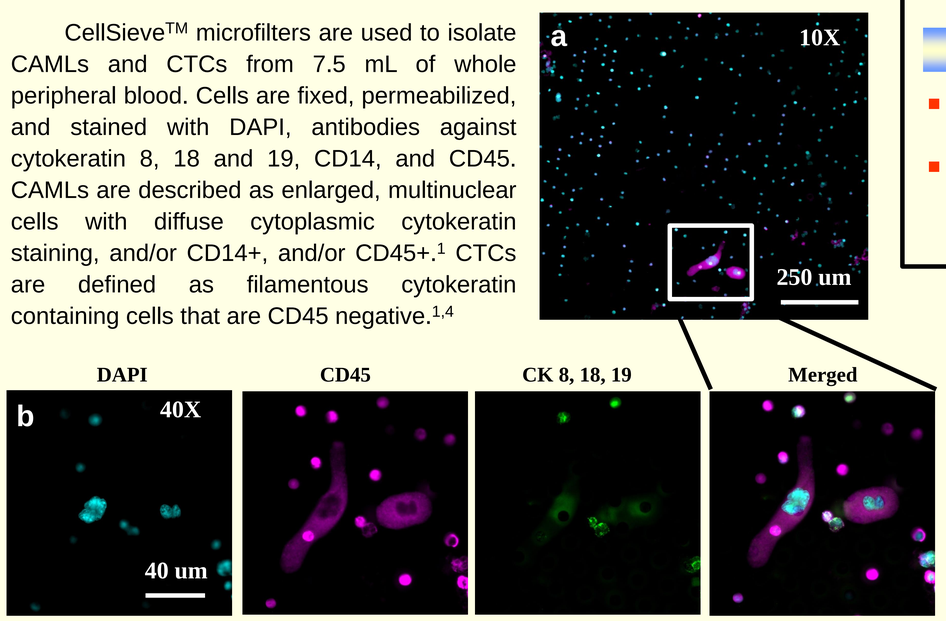


Figure 1. Isolation and identification of CAMLS by size and nuclear size
(a) CAMLs are easily identified under 10X magnification from a prostate patient
(b) Under 40X magnification the large polyploid nuclear structure can be seen (DAPI). These cells are positive for CD45 and weakly positive for cytokeratin

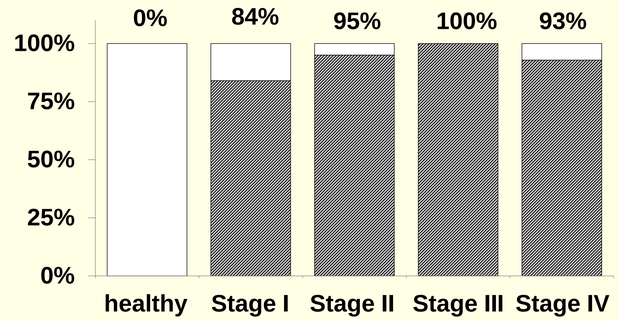
Study 1

MATERIALS & METHODS

Peripheral blood samples from 61 cancer patients were tested. Patients were, Stage I (n=25), Stage II (n=20), Stage III (n=2) and stage IV (n=14); from breast (n=8), pancreatic (n=22), lung (n=5) and prostate (n=26) cancers, newly diagnosed and untreated. Non-blinded blood samples from 30 healthy volunteers served as controls.

RESULTS

- CAMLs were found in 84% of patients with Stage I cancers, 95% with Stage II, 100% with stage III and 93% with stage IV; and 89% overall.
- By disease type, CAMLs were found in 77% of prostate, 100% of pancreatic, 80% of lung, and 100% of breast patient samples.
- CAMLs were not found in healthy control samples.



Sensitivity: 90% (CI95% 80-96%)
Specificity: 100% (CI95% 88-100%)
PPV: 100% (CI95% 94-100%)
NPV: 88-97% (CI95% 67-94%)

Study 2

MATERIALS & METHODS

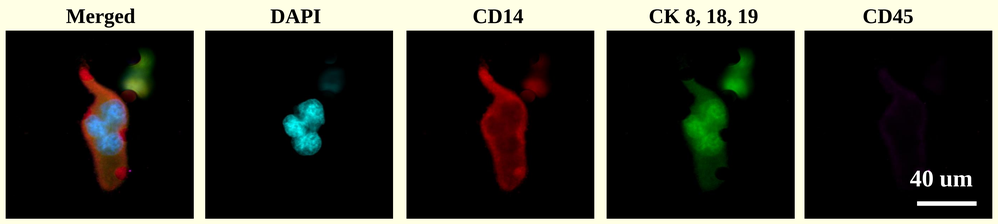
Double blinded peripheral blood samples from 20 patients with abnormal mammogram results were drawn at time of tissue biopsy and filtered at Creatv MicroTech. Following CAML analysis and pathological biopsy review, unblinding of biopsy data showed a distribution of malignant breast disease (n=10), benign (n=8), and atypical hyperplasia (AH) (n=2).

RESULTS

- CAMLs were found in 8 patients with malignant disease, 2 with benign disease, and 2 with AH.
- In this preliminary small pilot study the presence of CAMLs showed a potential to differentiate benign and malignant populations.

Sensitivity: 80% (CI95% 44-97%)
Specificity: 75% (CI95% 35-96%)
PPV: 80% (CI95% 44-97%)
NPV: 75% (CI95% 35-96%)

Figure 2. A 70 um CAML with large nuclear structure is shown. This CAML is positive for CD14, cytokeratin and CD45.



CONCLUSIONS

- Microfiltration captures CAMLs and CTCs regardless of surface marker expression.
- CAMLs can be used as a non-invasive blood biomarker to detect the presence of solid malignancies and potentially pre-malignant lesions.
- The presence of CAMLs in newly-diagnosed breast malignancies suggests their use as a biomarker for early breast cancer screening.
- In a double blind breast cancer study, CAMLs differentiated between benign and malignant breast disease.
- Preliminary data suggests the need for expanded cohorts including additional clinical samples with various disease states.

References

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