1st International Conference on Polyploid Giant Cancer Cells
February 16-17, 2024

Contact: dan@creatvmicrotech.com
301-983-1650

Polyploidization of cancer associated macrophages in blood circulation acts as a blood-based biomarker for screening invasive solid tumors

Daniel L. Adams[1], Steven H. Lin[2], Daniel Gironda[3], Raymond C. Bergan[4], Susan Tsai[5], Mohammed Aldakkak[5], Amy Kim[6], Kazuaki Chikamatsu[7], Masanori Hayashi[8], David M. Loeb[9], Cha-Mei Tang[10], Thai H. Ho[11], Jeff R. Marks[12]

[1]Creatv MicroTech, Monmouth Junction, NJ, [2]MD Anderson Cancer Center, Houston, TX, , [3]Department of Cancer Biology, Wake Forest School of Medicine, Winston-Salem, NC, [4]Eppley Institute, University of Nebraska Medical Center, Omaha, NE, [5]The Medical College of Wisconsin, Milwaukee, WI, [6]Johns Hopkins University, Baltimore, MD, [7]Gunma University, Maebashi, Japan, [8]Children's Hospital of Colorado, University of Colorado, Aurora, CO, [9]Albert Einstein College of Medicine, Bronx, NY, [10]Creatv MicroTech, Potomac, MD, [11]Mayo Cancer Clinic, Scottsdale, AZ, [12]Duke University Medical Center, Durham, NC

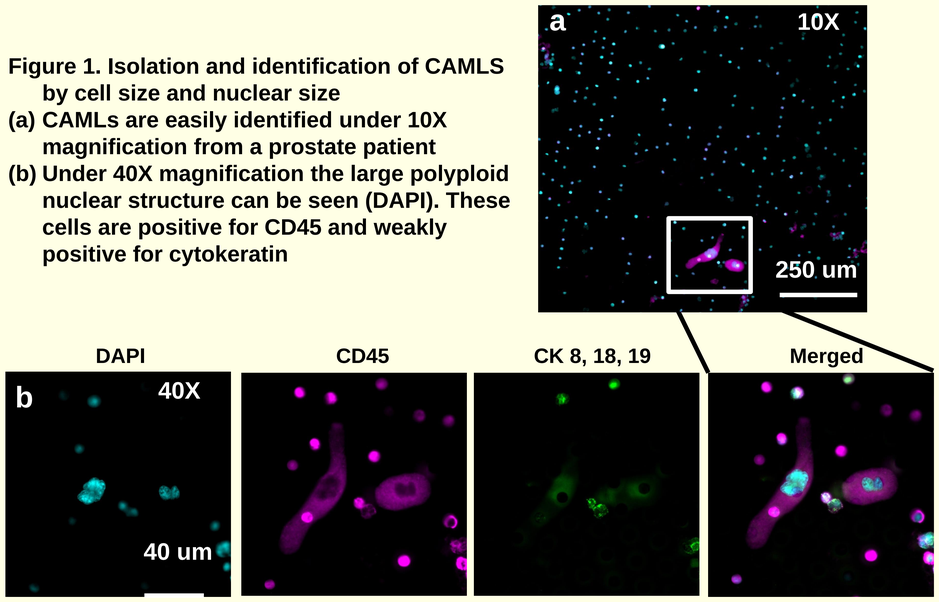
ABSTRACT

Monitoring peripheral blood is a simple, non-invasive method for isolating various cancer-associated circulating stromal cells (CStCs). A specific subtype of CStC, described as Cancer Associated Macrophage-Like cells (CAMLs), are polyploid tumor-myeloid hybrid cells that derive from an immunological response to invasive cancer emanating from tumors. Using a filtration method, we screened the peripheral blood of untreated newly diagnosed cancer patients with solid tumors (n=363) for CAMLs, patients with non-malignant diseases (i.e. lupus, benign cysts; n=53), and healthy controls (n=80). We found that CAMLs are highly prevalent in the blood of cancer patients, uncommon in non-malignant conditions, and absent in healthy individuals.

MATERIALS & METHODS

Anonymized peripheral blood was taken from 363 cancer patients with pathologically confirmed invasive malignancy [stage I (n=97), stage II (n=83), stage III (n=99), stage IV (n=84). Cancer types analyzed include lung (n=65), breast (n=60), prostate (n=60), pancreas (n=53), esophageal (n=30), renal cell (n=18), hepatocellular (n=18), colorectal (n=16), sarcoma (n=12), or other (n=31). Further, anonymized blood was taken from patients with untreated non-malignant conditions, i.e. benign breast masses (n=21), lupus (n=11), liver cirrhosis (n=5), benign prostatic masses (n=15), or viral infection (n=1), as well as healthy controls (n=80). CAMLs were isolated from whole blood by CellSieve™ microfiltration and defined as >25µm diameter polyploid cells by cytokeratin and/or CD45+CD14.

Figure 1. Isolation and identification of CAMLS by cell 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



INTRODUCTION

CAMLs are specialized myeloid polyploid cells transiting the circulation of patients with various types of solid malignancies and appearing in all stages of cancer[1-4]. However, while CAMLs are easy to identify by their large size and polyploid nucleus (Fig. 1), their expression of multiple heterogeneous markers have defied conventional characterization and have made study difficult. Size exclusion is a technique for isolating large cells from peripheral patient blood irrespective of their surface marker expression. CellSieve™ microfilters are size exclusion membranes which efficiently isolate CAMLs from whole blood, making it possible to study CAMLs in relation to malignant disease.

References

1. Adams DL, et al "Circulating giant macrophages as a potential biomarker of solid tumors." Proc Natl Acad Sci, 2014 111(9):3514-3519
2. Mu et al, Detection and Characterization of Circulating Tumor Associated Cells in Metastatic Breast Cancer" *Int. J. Mol. Sci.* 2016, *17*(10), 1665
3. Adams DL, et al, Circulating cancer-associated macrophage-like cells differentiate malignant breast cancer and benign breast conditions *CEBP*, 2016 25(7) 1037
4. Cristofanilli M, "Liquid Biopsies in Solid Tumors" *Humana Press*. 2017

RESULTS

- CAMLs were found in 83% of all cancer patients (~5.4 CAMLs/7.5mL blood)
  - 70% of Stage I, 86% Stage II, 86% Stage III, and 93% Stage IV patients (**Fig 2**).
- No CAMLs were found in any healthy controls. (**Fig 2**)
- CAMLs were found in 30% of benign conditions. (**Fig 2**)
  - Including: 30% of benign breast masses, 18% of lupus patients, 13% of benign prostatic hyperplasia and 0% of liver cirrohosis.
  - 8% (n=4) of "benign" patients had histology's with increased risk of cancer
- CAML sensitivity in cancer vs healthy was 84% (CI95 79-87%), specificity=100% (CI95 95-100%), PPV=100% (CI95 99-100%), NPV=57% (CI95 51-63%) (**Fig 3**)
- CAML sensitivity in cancer vs benign was 84% (CI95 79-87%), specificity=77% (CI95 64-87%), PPV=96% (CI95 94-97%), NPV=41% (CI95% 34-47) (**Fig 3**).

Figure 2. Percentage of patients with CAMLs by Stage or Cancer

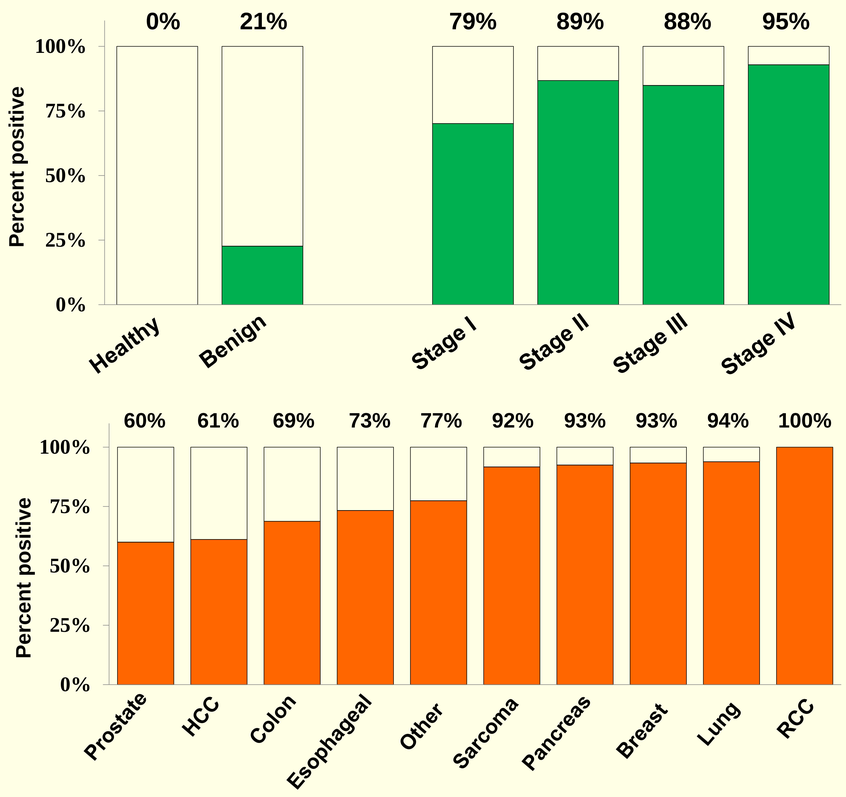
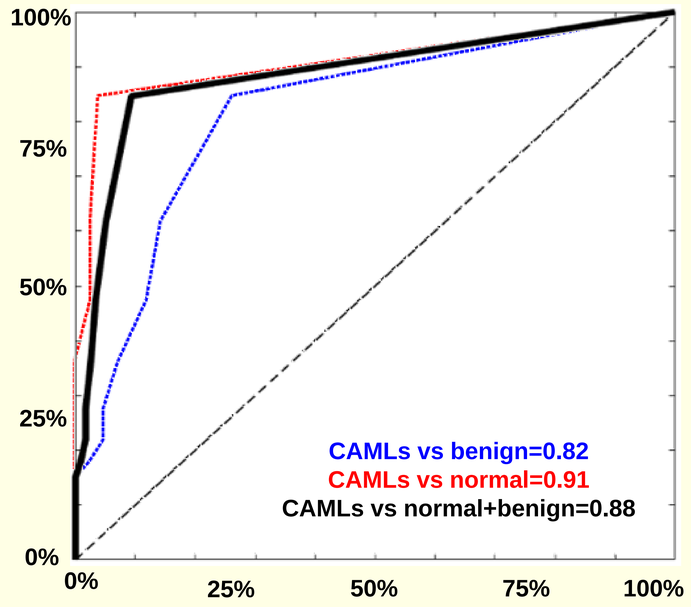


Figure 3. AUC chart-patients with carcinoma vs healthy control or carcinoma vs benign



CONCLUSIONS

- Using combination of clinical studies, we found CAMLs (a Circulating Stromal Cell subtype) are a sensitive blood biomarker specific to patients with confirmed malignancy.
- CAMLs were uncommon in non-malignant conditions and absent in healthy individuals
- CAMLs appear to be a sensitive and specific blood based biomarker for persons with solid tumor malignancies

Funding Sources

This work was supported by a grant R01-CA154624 and UO1CA214183 from the National Cancer Institute and the U.S. Army Research Office (ARO) and the Defense Advanced Research Projects Agency (DARPA) (W911NF-14-C-0098). The content of the information does not necessarily reflect the position or the policy of the US Government.