Molecular Med Tri-Con San Francisco, CA March 10-15, 2019

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

Real time monitoring of solid tumor progression using circulating stromal cells in early and late stage disease

Daniel L Adams[1], Raymond C Bergan[2], Martin J. Edelman[3,4], Stuart S. Martin[4], Rena Lapidus[4], Saranya Chumsri[5], Cha-Mei Tang[6], Steven H. Lin[7]

[1] Creatv MicroTech, Monmouth Junction, NJ 08852, [2] OHSU Knight Cancer Institute Portland OR 97239, [3]Fox Chase Cancer Center, Philadelphia, PA 19111, [4]University of Maryland School of Medicine, Baltimore, MD 21201, [5]Mayo Clinic Jacksonville, FL 32224, [6]Creatv MicroTech, Rockville, MD 20850, [7]MD Anderson Cancer Center, Houston, TX 77030

ABSTRACT

Numerous blood based biomarkers (PSA, CEA, CA125) are currently used to track real time progression of disease in parallel with imaging. However while many blood biomarkers exist for use in cancer, they are specific to cancer type (i.e. PSA to prostate and CEA to colon) and may not appear in all diseased individuals. Recently cancer associated macrophage-like cells (CAMLs), a circulating stromal cell subtype, were identified in a variety of solid cancer types and were observed increasing in size during progressive disease. To assess whether CAML enlargement is an indicator of progression/response, we tracked CAML growth/shrinkage in a pilot group of patients (n=34). Blood was drawn from patients with lung, prostate, or breast cancer over a 3 month period, baseline through 2 treatment cycles, followed by monitoring over 2 years. These data suggest that morphological assessment of CAMLs (growth) appear to parallel cancer progression, or response to treatment, in a variety of solid tumors.

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

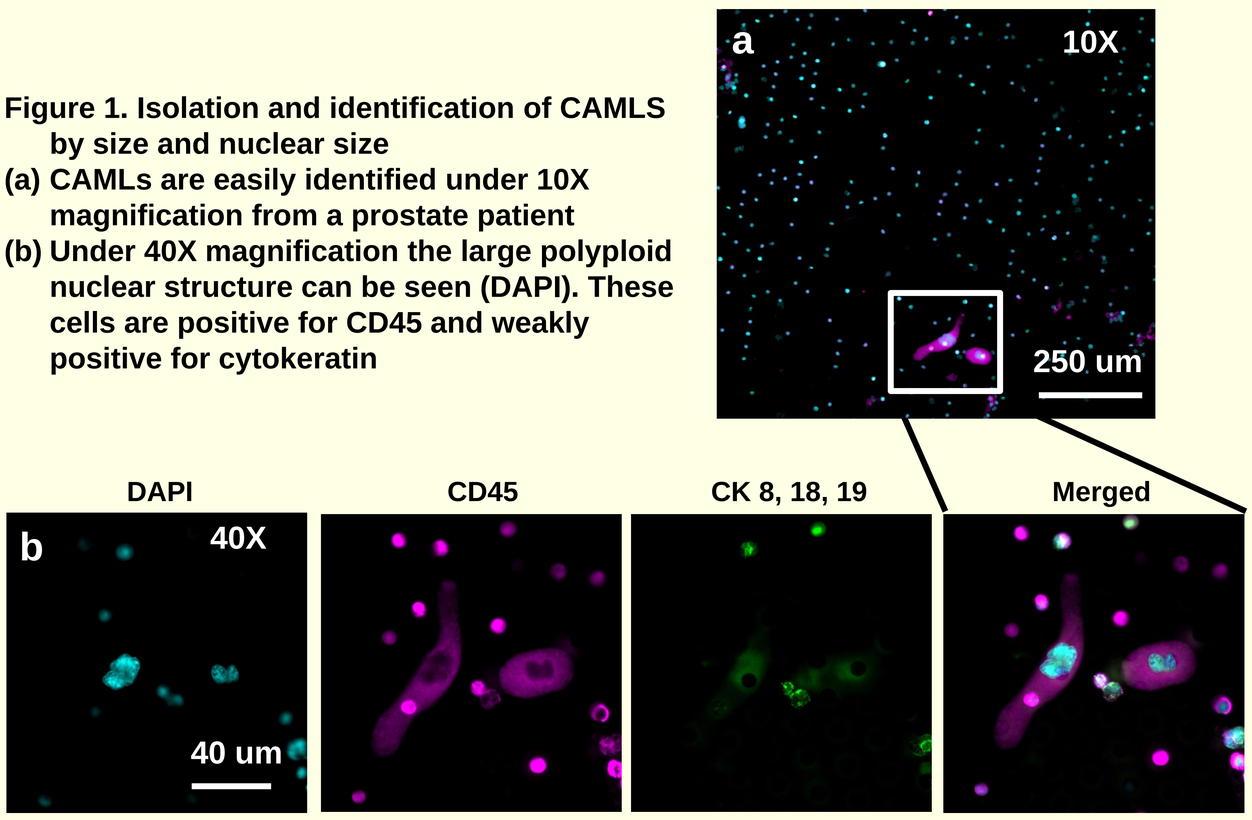


Figure 3. Analysis of PFS at BL vs FU2 based on size of CAMLs

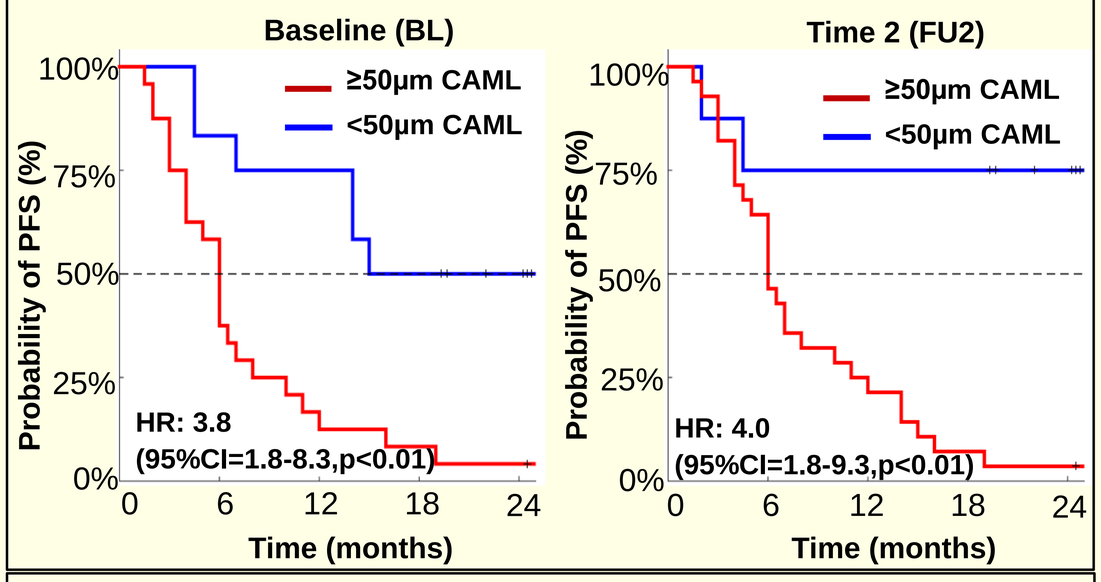
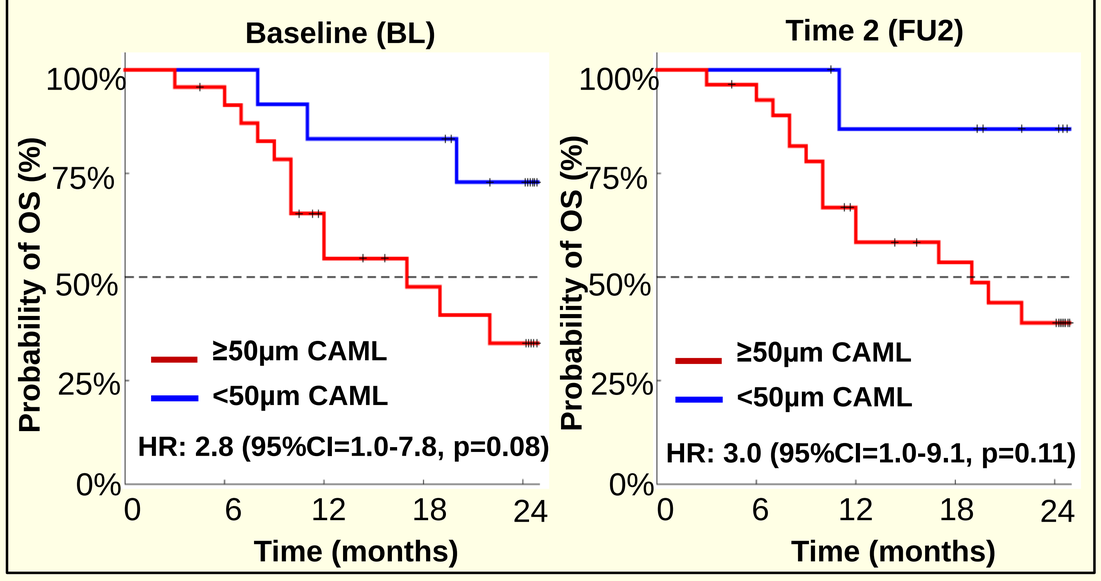


Figure 4. Analysis of OS at BL vs FU2 based on size of CAMLs



INTRODUCTION

CAMLs are specialized myeloid polyploid cells transiting the circulation of patients in various types of solid malignancies and appearing in all stages of cancer[1-4]. While CAMLs are easy to identify by their large size and polyploid nucleus, they appear to present as stem cell like phenotype with multiple heterogeneous epithelial, myeloid, and endothelial markers.

Size exclusion is the only known technique for isolating large cells from peripheral patient blood irrespective of their surface markers. CellSieve™ microfilters are size exclusion membranes which efficiently isolate CAMLs and circulating tumor cells (CTCs) from whole blood, making it possible to study both cell types in relation to malignant disease[1-4].

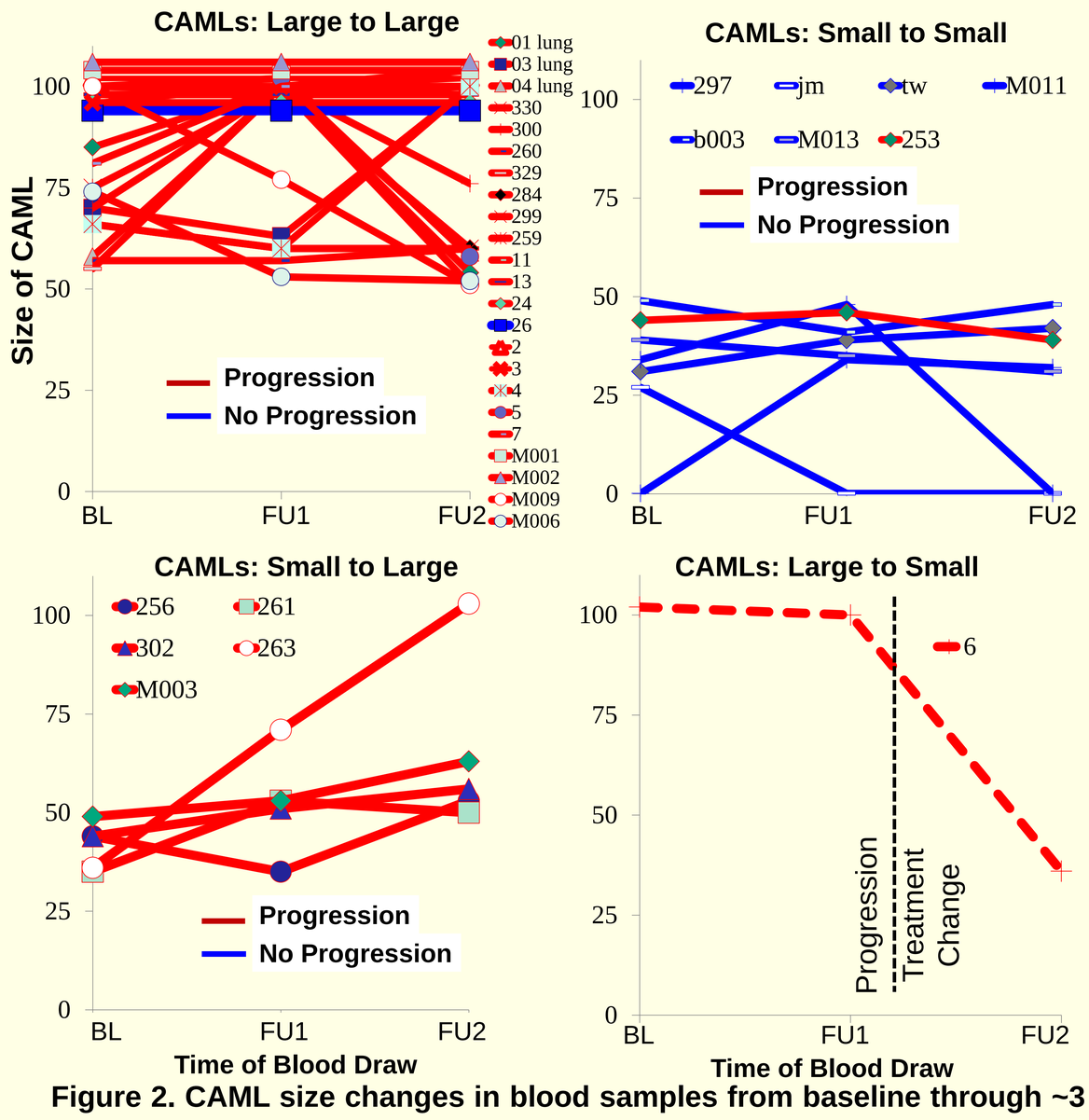


Figure 2. CAML size changes in blood samples from baseline through ~3 months of treatment based on change in largest CAML size

MATERIALS & METHODS

A prospective multi-institutional study used anonymized peripheral blood samples from 34 cancer patients undergoing therapy [stage I (n=2), II (n=3), III (n=8) & IV (n=21)] with breast (n=10), lung (n=16), & prostate (n=8). Samples were taken prior to therapy (BL), at ~1 month (FU1) follow up and a ~3 month (FU2) follow up, after induction of therapy. Blood was processed by the CellSieve™ microfiltration technique at 4 institutions and stained for cytokeratin 8, 18 & 19, CD14 and CD45. After identification and quantification CAMLs were measured based on hyperploidy and cell size.

RESULTS

- CAMLs were found in 97% of cancer patients at BL and 91% at FU time points
- Over 2 years 7 patients showed no clinical disease progression (blue), while 29 patients had observable clinical disease progression (red).
- Of the 7 patients with no progression (blue), 1 had CAMLs of ≥50µm at all time points while 6 had only small CAMLs
- Of the 29 patients that progressed,
  - 22 patients had ≥50µm CAMLs at all time points;
  - 5 patients had <50µm CAMLs at BL which increased in size by FU2;
  - 1 patient had ≥50µm CAMLs at BL that decreased by FU2
  - 1 patient had small CAMLs at all time points

CONCLUSIONS

- CAML enlargement after baseline indicates shorter PFS in a variety of cancer types.
- Monitoring CAML changes during treatment correlates to ongoing progression, or response
- This pilot study suggests that CAMLs have the potential to monitor progression/regression of malignancy in real time and suggests the need for larger validation studies.

Funding Sources

This work was supported by a Maryland (TEDCO) MTTCF award, grant R01-CA154624 from the National Cancer Institute, grant KG100240 from the Susan G. Komen Foundation, 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.

References

1. Adams DL, et al "Circulating giant macrophages as a potential biomarker of solid tumors." Proc Natl Acad Sci, 2014 111(9):3514
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. Cristofanilli M, "Liquid Biopsies in Solid Tumors" *Springer Intl Publish.* 2017