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Multiplex phenotyping of circulating cancer associated macrophage-like cells in patients with solid tumors

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

Daniel Adams[1], R. Katherine Alpaugh[2], Thai Ho[3], Steven H. Lin[4], Jeffrey R. Marks[5], Raymond Bergan[6], Stuart S. Martin[7], Saranya Chumsri[7,8], Cha-Mei Tang[9], Massimo Cristofanilli[10]

[1]Creatv MicroTech, Inc., Monmouth, NJ 08852, [2]Fox Chase Cancer Center, Philadelphia, PA 19111, [3]Mayo Clinic Cancer Center Phoenix, AZ 85054, [4] MD Anderson Cancer Center, Houston, TX 77030, [5]Duke University Medical Center, Durham, NC 27710, [6]OHSU Knight Cancer Institute Portland OR 97239, [7]University of Maryland School of Medicine, Baltimore, MD 21201, [8]Mayo Clinic Cancer Center Jacksonville, FL 32224, [9]Creatv MicroTech, Inc., Potomac, MD 20854, [10]Northwestern University, Chicago, IL 60611

ABSTRACT

Cancer associated macrophage-like cells (CAMLs) are a recently identified cancer specific giant cell circulating in the blood of patients with solid tumors. However, since their discovery few studies have been done to elucidate their lineage or phenotypic identity. The difficulty in classifying CAMLs is been exemplified by recent publications describing their expression of multiple heterogeneous markers that defy conventional identification. Recently, we described a restaining method (QUAS-R) to screen individual rare cells using an array of up to 15 biomarkers. We used this method to screen CAMLs isolated from 152 cancer patient samples in 4 types of solid tumors to classify CAMLs by phenotypic immunostaining. These data suggest that CAMLs are a morphologically diverse and phenotypically heterogeneous population of cancer specific giant cells with overlapping myeloid, epithelial, and endothelial phenotypes.

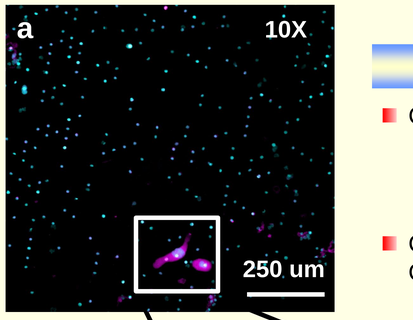
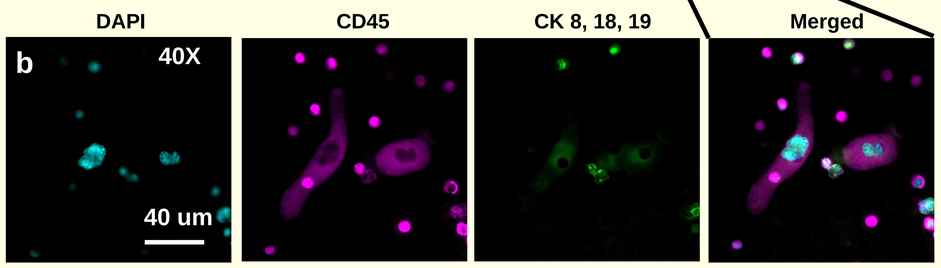


Figure 1. Isolation and identification of CAMLS by size and nuclear size
(a) CAMLs are easily identified under 10X magnification from a prostate patient
(b) Under 40X magnification the large polyploid nuclear structure can be seen (DAPI). These cells are positive for CD45 and weakly positive for cytokeratin

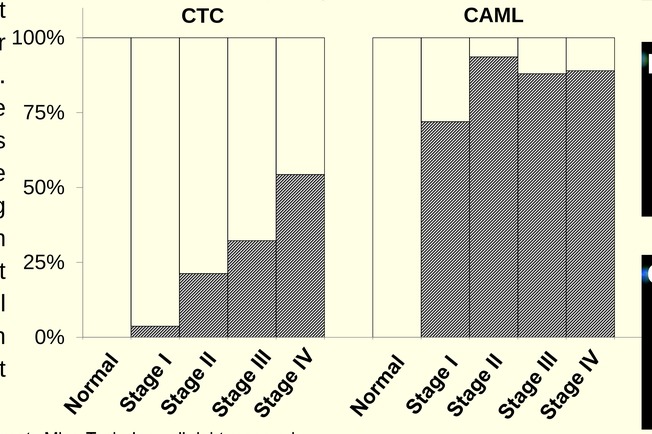


INTRODUCTION

CAMLs are specialized myeloid polyploid cells transiting the circulation of patients with various types of solid malignancies and appearing in all stages of cancer[1,2]. However, while CAMLs are easy to identify by their large size and polyploid nucleus, their expression of multiple heterogeneous markers have defied conventional characterization and have made study difficult using most isolation technologies.

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 and circulating tumor cells (CTCs) from whole blood, making it possible to study both cell types in conjunction with and in relation to malignant disease[1-4].

Figure 2. Percentage of patients positive for CAMLs and CTCs by stage



MATERIALS & METHODS

This multi-institutional study used peripheral blood samples from 152 cancer patients (stage I-IV) from breast (n=42), lung (n=39), renal cell carcinoma (n=36) and prostate (n=35). Blood was processed by the CellSieve™ microfiltration technique at 5 institutions and stained for cytokeratin 8, 18 & 19, EpCAM, and CD45. After identification/imaging, the QUAS-R (Quench, Underivatize, Amine-Strip and Restain) technique was used to quench fluorescence signal of cells and then restain with CD38, CD24, CD14, CD61, CD41, CD11c, CD11b, CD68, CD206, CD163, CD31, CD146, CD202, VEGFR1, VEGFR2, CD144, CD133, CD44, CD133, CD34, HGFR, Vimentin, or CXCR4.

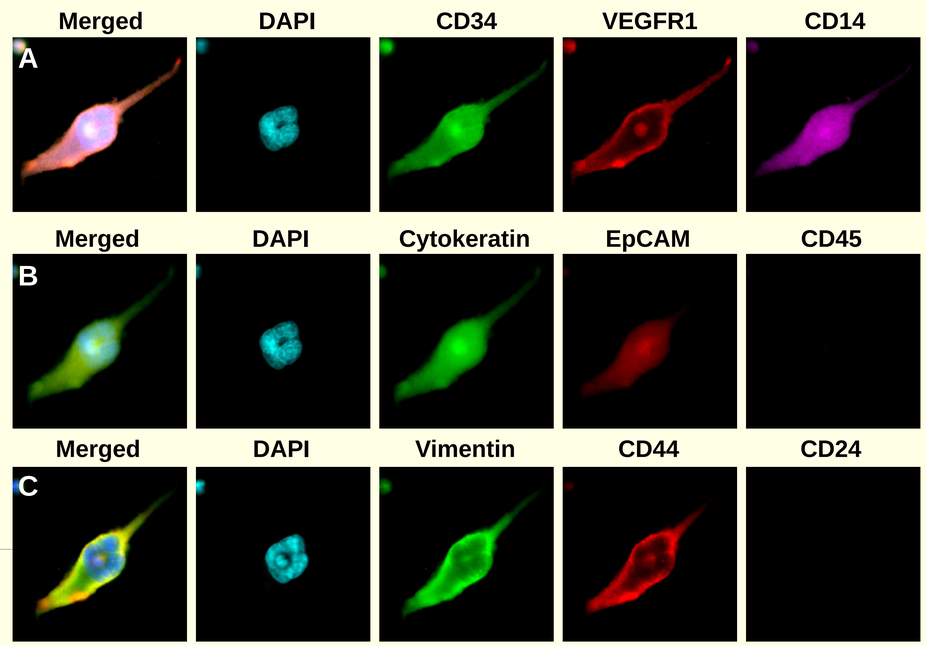
RESULTS

- CAMLs were found in 86% of cancer patients (n=131/152), but in none of the healthy control samples
  - CAMLs were commonly found in stage 1 (71%), stage 2 (94%), stage 3 (88%) to stage 4 (88%) (Fig. 2).
  - Breast cancer had the most CAMLs per sample (14.1 cells/7.5mL), followed by prostate (6.8), renal cell carcinoma (4.9) and lung (3.2).
- CD31 was most the prevalent, marker found on 96% of CAMLs, followed by cytokeratin (89%), CD14 (87%), CD41 (78%), CD61 (75%), CD45 (74%), etc. (Figure 2)

CONCLUSIONS

- CAMLs express overlapping phenotypes from a variety of lineages i.e. macrophage (CD14/CD68/CD11c), epithelial (cytokeratin/EpCAM), endothelial (CD146/TIE-2) and megakaryocyte (CD41/CD61).
- Multi-phenotypic subtyping can identify and subtype CAMLs from cancer patients with multiple solid tumor types.
- CAMLs cannot be grouped into any known cell subtype but seem to represent a variably heterogeneous population of myeloid lineage with stem cell like phenotypes.

Figure 3. Representative example of QUAS-R testing 9 subtyping markers on one CAML



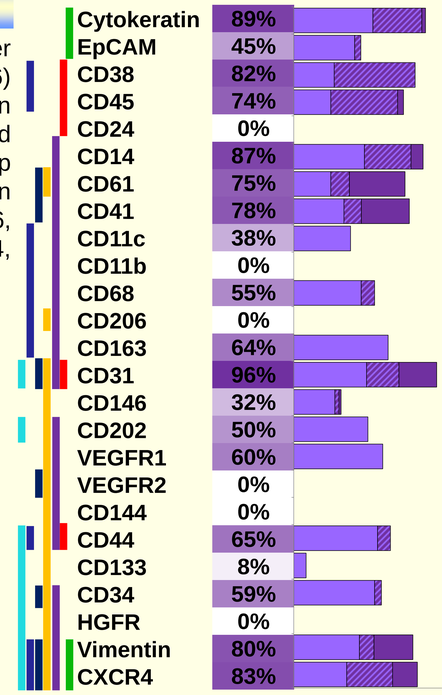


Figure 4. Percentage of CAMLs positive, and the biological utility and expression level of various phenotypes on CAMLs.

References

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