EDRN, Bethesda, MD, September 8-10, 2014

Cancer Associated Macrophage-like Cells in Baseline Blood Samples of Cancer Patients Indicate Malignant Disease

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

Daniel L. Adams[1], Stuart S. Martin[2] , Monica Charpentier[2], Saranya Chumsri[2], Rena Lapidus[2], Raymond C. Bergan[3], Irene M. Ogden[3], R. Katherine Alpaugh[4], Susan Tsai[5], Massimo Cristofanilli[6], Olga V. Makarova[7], Peixuan Zhu[8], Shuhong Li[8], Platte Amstutz[9], Cha-Mei Tang[8,9]

[1]Creatv MicroTech, Inc., Monmouth Junction, NJ; [2]University of Maryland Baltimore, MD; [3]Northwestern University, Chicago, IL, [4]Fox Chase Cancer Center, Philadelphia, PA; [5]The Medical College of Wisconsin, Milwaukee, WI; [6]Thomas Jefferson University Hospital, Philadelphia, PA; [7]Creatv MicroTech, Inc., Chicago, IL [8] Creatv MicroTech, Inc., Rockville, MD; [9]Creatv MicroTech, Inc., Potomac, MD

ABSTRACT

Use of peripheral blood as a "liquid biopsy" allows for the analysis of various cancer associated circulating cells, including circulating tumor cells (CTCs) and cancer associated macrophage-like cells (CAMLs). CTCs are malignant cells derived from solid tumors that enter the circulatory system, after breaking off from the original tumor site; while CAMLs are phagocytic myeloid cells, thought to be an immunological response to the tumor. Using precision microfilters, we isolated and identified both CAMLs and CTCs from a variety of cancer patients. We identified CTCs using the classical definition of filamentous cytokeratin and CD45 negativity, while identifying CAMLs by their large size, 25 µm to 300 µm, diffused cytokeratin, and multinucleated structure. It has been discussed that both cell types can be used as an indication of malignant disease in multiple solid tumors. Here we analyzed the peripheral blood of untreated newly diagnosed cancer patients to ascertain the prevalence of CTCs and CAMLs; and supply evidence that CAMLs are a highly prevalent biomarker which might be used for the early detection of solid tumors.

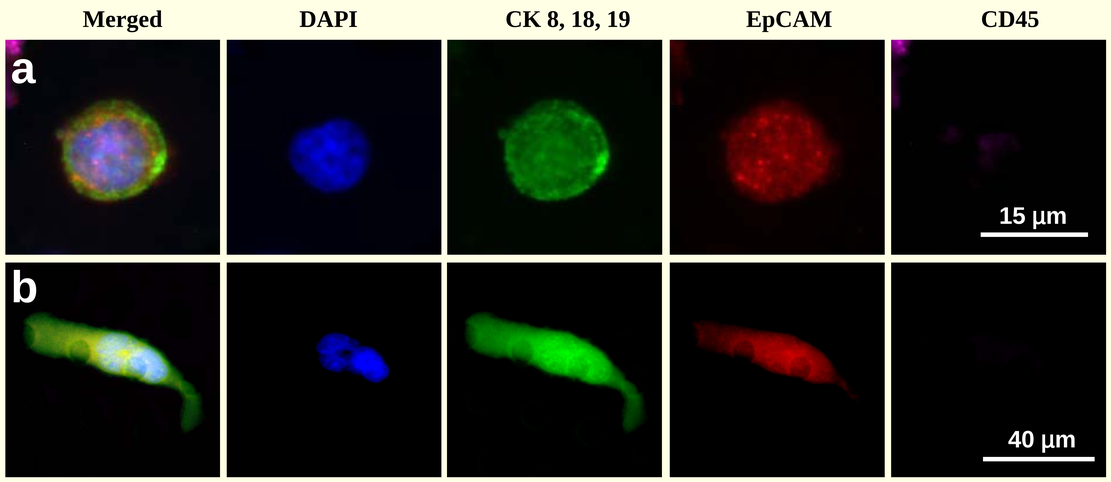


Figure 1. Examples of a CTC and a CAML. (a) CTC with single nucleus, high cytokeratin filamentation and high EpCAM expression (approx. 15 µm in length). (b) CAML with enlarged multinucleation, diffuse cytokeratin staining and some EpCAM expression (approx. 80 µm in length).

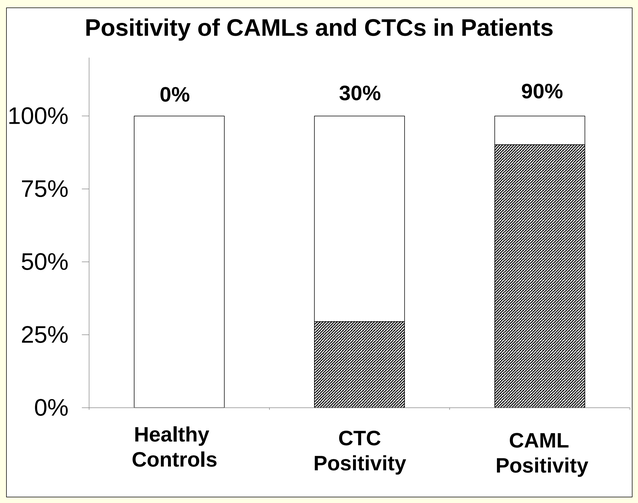
INTRODUCTION

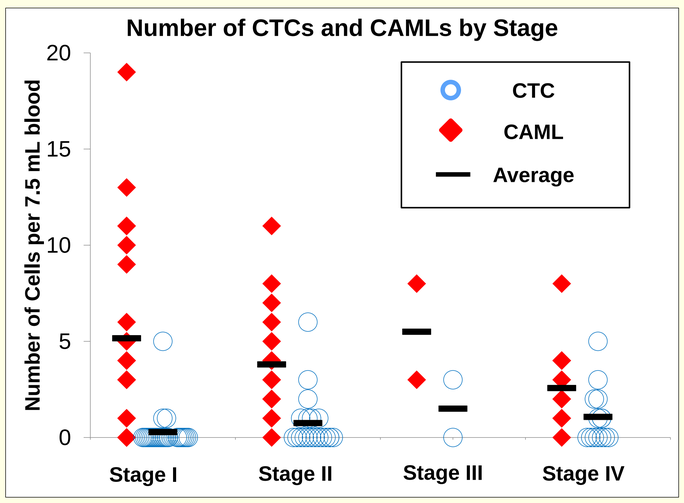
CTCs have been shown to be an indicator of malignant disease, used to monitor therapy response and predict outcomes in late stage patients.[1-4] However, CTCs are not common in early stage disease and are found in low frequencies in a number of cancers, including lung and pancreatic cancers. CAMLs are immunological cells which have been shown to be present in all stages of cancer, and in multiple cancer types, but have remained largely unstudied.

CellSieve™ microfilters are lithographically fabricated membranes with high porosity, precise pore dimensions, and regular pore distribution[3-4]. We previously reported that CellSieve™ rapidly and efficiently isolates both CAMLs and CTCs from whole peripheral blood, showing it is possible to study both cell types in conjunction with and in relation to malignant disease. [3-4]

MATERIALS & METHODS

Blinded peripheral blood samples from 61 cancer patients were tested at initial clinical diagnosis, from a variety of cohorts including stage I (n=25), II (n=20), III (n=2), and IV (n=14) patients with breast (n=8), pancreatic (n=22), lung (n=5) and prostate (n=26) cancers; all from newly diagnosed and untreated patients. The study included 30 healthy controls with no known malignant disease. CellSieve™ microfilters were used to isolate both CTCs and CAMLs from 7.5 mL of whole peripheral blood. The pore size of CellSieve™ is 7 µm, capable of isolating both CTCs and CAMLs based on size. CTCs and CAMLs collected by CellSieve™ were fixed, permeabilized, and stained with DAPI, antibody cocktail against cytokeratin 8, 18 and 19, EpCAM, and CD45. CAMLs were defined as enlarged, multinuclear cells with diffuse cytoplasmic cytokeratin, commonly CD45 positive. CTCs were defined as filamentous cytokeratin cells that are not positive for CD45.





RESULTS

- CAMLs and CTCs were not found in healthy individuals (n=30).
- CAMLs were found in 90% of all 61 patients regardless of cancer type or stage
- CAMLS averaged 4.2 cells per sample of all 61 patients
- CTCs were found in 30% of the same patient cohort, averaging 0.7 cells per sample.
- CAMLs were found in 84%(stage I), 90%(stage II), 100%(stage III) and 93%(stage IV)
- CTCs were found in 12% (stage I), 35% (stage II). 50% (stage III) and 50% (stage IV)
- CAMLs have vacuoles containing the same biomarkers found at primary tumor sites.

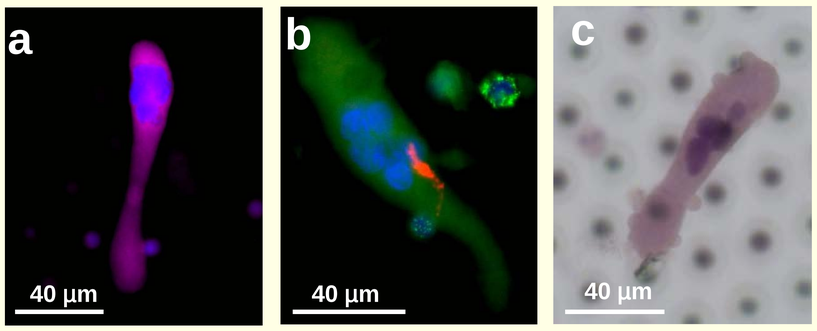


Figure 2. Attributes of CAMLs. (a) Single ~100 µm CAML with intense CD45 (violet) staining (b) Single CAML from a prostate patient with cytokeratin signal (green) and a vacuole positive for prostate specific membrane antigen (red) (c) H&E stain of a CAML. Scale bars, 40 µm.

CONCLUSIONS

- We present evidence that CAMLs can be found in the blood of most cancer patients isolated by precision microfilters.
- The presence of CAMLs suggests malignant disease
- The high frequency and high specificity of CAMLs suggest a their possible use as a biomarker for early detection of solid tumors.
- Non-malignant cells should be considered when analyzing fluids in a "liquid biopsy" setting
- Additional prospective studies with larger cohorts are called for
- Proteomic and genomic characterization of CAMLs may allow for identification of cancer type during initial disease screening.

References

1. Cristofanilli M, et al, "Circulating tumor cells, disease progression, and survival in metastatic breast cancer." N Engl J Med 351:781-91, 2004
2. Plaks, V., Koopman, CD, & Werb, Z.,"Circulating Tumor Cells." Science 341(6151): 1186-1188.
3. Adams DL, et al, "The systematic study of circulating tumor cell isolation using lithographic microfilters." RSC Advances 4:4334-4342, 2014
4. Adams DL, et al "Circulating giant macrophages as a potential biomarker of solid tumors." Proc Natl Acad Sci, 111(9):3514-3519, 2014