Tri-CON 2015, San Francisco, February 15-20, 2015

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

Cancer Associated Macrophage-Like cells in the blood of cancer patients

Daniel L. Adams[1], Stuart S. Martin[2] , Monica Charpentier[2], Saranya Chumsri[2], Martin Edelman[2], Raymond C. Bergan[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[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

Using a "liquid biopsy" for recovering Circulating Tumor Cells (CTCs) has revealed an accompanying circulating macrophage subset that may be a highly sensitive blood based biomarker for solid tumors. This newly discovered cell was defined as circulating cancer associated macrophage-like cell (CAML) and has been identified as a specialized cancer specific blood monocyte (CD14+)[1]. We studied the peripheral blood of 100 cancer patients with breast, prostate, pancreatic or lung cancer; stages including I-IV; to study the prevalence of this cell in relation to CTCs. As CTCs and CAMLs appear numerically independent (R^2=0.13, p=0.21), we looked to see how both CAMLs and CTCs might be used as blood based biomarkers for tracking malignant disease. Here, we supply evidence that this specialized circulating blood cell can be used in conjunction with CTCs to sequentially track cancer treatment and provide clinical utility in patients with solid tumors using non-invasive sampling techniques.

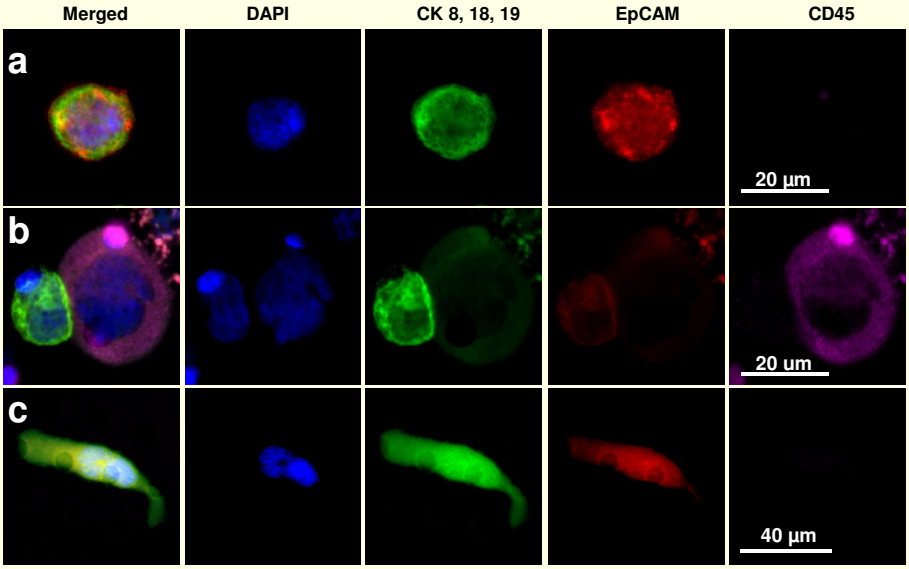
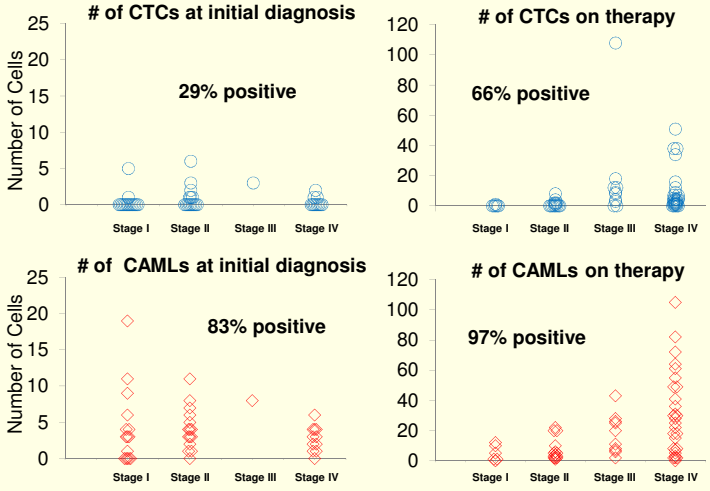


Figure 1. Examples of CTCs and CAMLs. (a) CTC with single nucleus, high cytokeratin filamentation and EpCAM expression. (b) CAML with enlarged multinuclear structure, diffuse cytokeratin, and CD45 expression, bound to a CTC. (c) CAML with enlarged multinuclear structure, some EpCAM expression and no CD45 expression.

RESULTS

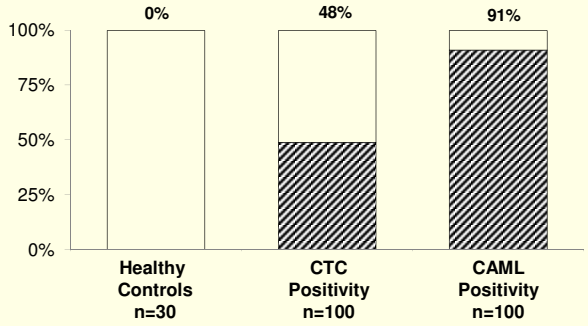
- CAMLs were found in 96% of patients with Stage III/IV cancer, 88% with Stage I/II and 91% of patients overall, regardless of cancer type.
- CTCs were found in 48% of the total sample set.
- CAMLs were found in 89% (lung), 79% (prostate), 97% (pancreatic), and 97% (breast).
- CAML and CTC number both seemed to correlate with treatment and stage of cancer.



MATERIALS & METHODS

Peripheral blood samples from 100 cancer patients were provided by University of Maryland, Greenebaum Cancer Center, Northwestern University, The Medical College of Wisconsin and Fox Chase Cancer Center. The patient distribution included Stage I (n=21), Stage II (29), Stage III (11), Stage IV (n=39); breast (n=31), pancreatic (n=31), lung (n=9), and prostate (n=29) cancers. We included newly diagnosed/untreated patients (n=42) as well as patients undergoing systemic therapies (n=58). The study included 30 healthy subjects. CellSieve™ microfilters were used to isolate CTCs and CAMLs from 7.5 mL of whole blood. Samples were diluted in a pre-fixation solution and filtered through the microfilters. CTCs and CAMLs collected by this size exclusion technique were fixed, permeabilized, and stained with DAPI, an antibody cocktail against cytokeratins 8, 18 & 19 (FITC), EpCAM (PE), and CD45 (Cy5). CAMLs were defined as large, multinuclear cells with diffuse cytoplasmic cytokeratin. CTCs were defined as filamentous cytokeratin cells that were CD45 negative.

Figure 2. Positivity of CAMLS and CTCs in Cancer Patients



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 all disease stages and are found in low frequencies in a number of cancers, including lung and pancreatic.

CAMLs are immunological cells which have been shown to be present in all stages of cancer, are reactive to cancer treatments, and are found in multiple cancer types. However, these cells 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, making it possible to study both cell types in conjunction with and in relation to malignant disease. [3-4]

CONCLUSIONS

- Highly differentiated monocytic cells, classified as circulating cancer associated macrophage-like cells transit the blood of most cancer patients.
- CAMLs may be used as a liquid biopsy for early detection and sequential tracking of cancer.
- High prevalence of CAMLs implies that complicated and minimally understood cellular interactions exist in the blood.
- CAMLs should be considered when evaluating blood from cancer patients.
- Detection, characterization and monitoring of multiple cellular components present in the blood is an essential element in proper characterization of solid tumors.

References

1. Adams DL, *et al.*, "Circulating giant macrophages as a potential biomarker of solid tumors." Proc Natl Acad Sci, 111(9):3514-3519, 2014
2. Cristofanilli M, et al., "Circulating tumor cells, disease progression, and survival in metastatic breast cancer." N Engl J Med 351:781-91, 2004
3. Plaks, V., *et al.*,"Circulating Tumor Cells." Science 341(6151): 1186-1188
4. Adams DL, *et al.*, "Cytometric Characterization of Circulating Tumor Cells Captured by Microfiltration and Their Correlation to the CellSearch® CTC Test." Cytometry 2014. doi: 10.1002/cyto.a.22613