ASCO, Chicago, IL May 29- June 2, 2015 **ABSTRACT #11095**

Contact:: cmtang@creatvmicrotech.com

Cancer associated macrophage-like cells correlate with systemic therapy and pathological stage in numerous malignancies

Daniel Adams[1], Raymond C. Bergan[2], Stuart S Martin[3], Saranya Chumsri[3,4], Monica Charpentier[3], Rena Lapidus[3], R. Katherine Alpaugh[5], Massimo Cristofanilli[6], Susan Tsai[7],Cha-Mei Tang[8], Martin J. Edelman[3]

[1]Creatv MicroTech, Inc., Monmouth Junction, NJ 08852; [2]Knight Cancer Institute, Portland, OR 97239, [3]University of Maryland Greenebaum Cancer Center, Baltimore, MD 21201; [4]Mayo Clinic Cancer Center, Jacksonville, FL 32224; [5]Fox Chase Cancer Center, Philadelphia, PA 19111; [6]Thomas Jefferson University Hospital, Philadelphia, PA19107; [7]Medical College of Wisconsin, Milwaukee, WI 53226; [8]Creav MicroTech, Inc., Potomac, MD 20854

ABSTRACT

Blood based testing can be used as a non-invasive method to recover and analyze Circulating Tumor Cells (CTCs) from the blood of cancer patients for numerous clinical implications. Furthermore, we recently published preliminary data on the presence of an additional cell-based cancer biomarker, Cancer Associated Macrophage-Like cells (CAMLs). These cells were present in a variety of malignancies and were used to track both therapy response and cancer progression (Adams et al., PNAS 2014). This report is a continuation of that study, to identify and track CAMLs, with an emphasis on correlating pre-surgical clinical assessment and pathological confirmation from baseline samples.

INTRODUCTION

CAMLs are specialized myeloid cells transiting the circulation of patients in all stages of cancer. They are responsive to cancer treatment and are found in multiple cancer types.[1,2] However, although previously observed by numerous groups, these cells have remained largely unstudied, and their clinical and biological value in malignancies remains uninvestigated.

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 capable of rapidly and efficiently isolating both CAMLs and CTCs from whole blood, making it possible to study both cell types in conjunction with and in relation to malignant disease. [1-4]

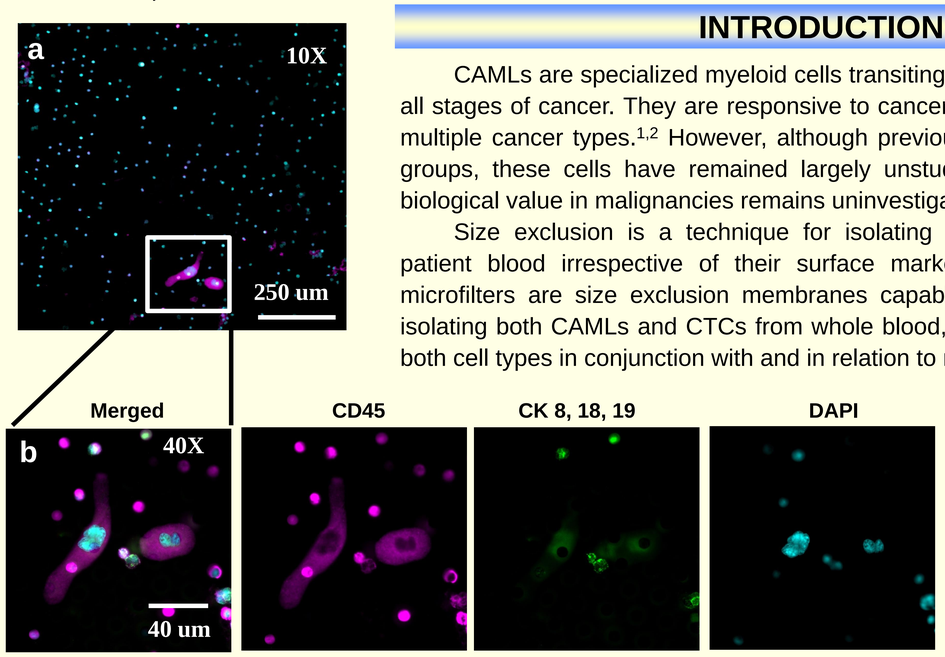


Figure 1. Isolation and identification of CAMLS by size and nuclear size (a) CAMLs are easily identified under 10X magnification from a prostate patient (b) Under 40X

MATERIALS & METHODS

This multi-institutional prospective pilot study consisted of 105 patient samples: Stage I-IV; breast (n=34), prostate (n=25), pancreatic (n=39) and lung (n=7). Evaluators were blinded to the source and stage of the samples. Additionally, 30 non-blinded healthy controls with no known malignant disease were analyzed. 7.5 mL of whole blood was collected, filtered by the CellSieve™ microfiltration assay, and stained with DAPI, CK 8/18/19, EpCAM, CD14 and CD45. CAMLs were enumerated and identified as large multinucleated circulating myeloid cells. (e.g. CD14+). We report CAML numbers at initial blood draw after pre-surgical clinical assessment in relation to healthy controls and compare with the patient's subsequent pathological staging confirmation.

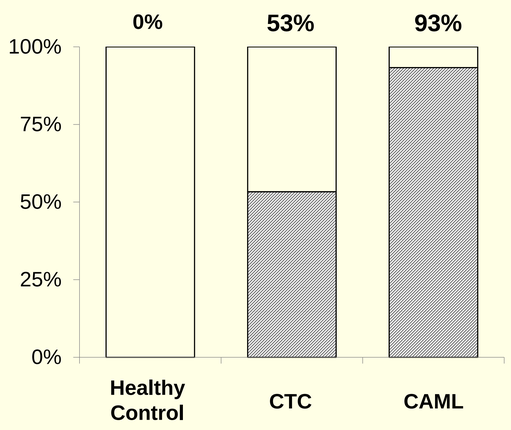
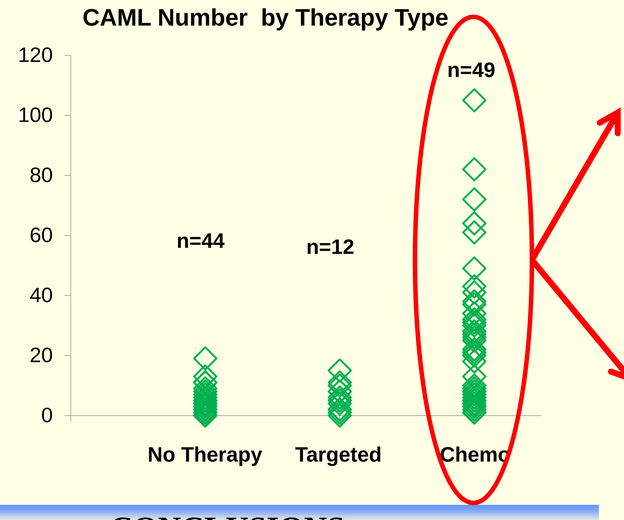


Figure 2. Positivity of CTC and CAMLs in patients with breast, prostate, pancreatic, and lung cancers

RESULTS

- CAMLs were identified in 98/105 samples (93%), ranging from 0-105 CAMLs per 7.5 mL blood sample.
- No CAMLs were found in 30 healthy controls [Sensitivity=93% (CI95% 87-97%) and Specificity=100% (CI95% 88-100%)].
- CAML number appeared dependent on both presence of malignancy and therapy type.
- For patients undergoing chemotherapy, the number of CAMLs had a **weak association with pre-surgical clinical assessment**; stage I (14.9 per sample), Stage II (13.7), Stage III (18.3), Stage IV (37.8); R^2=0.74.
- For patients undergoing chemotherapy, the number of CAMLs were **exponentially correlated with final pathological confirmation**; stage I (3.2), Stage II (7.1), Stage III (14.6), Stage IV (35.1); R^2=0.99.



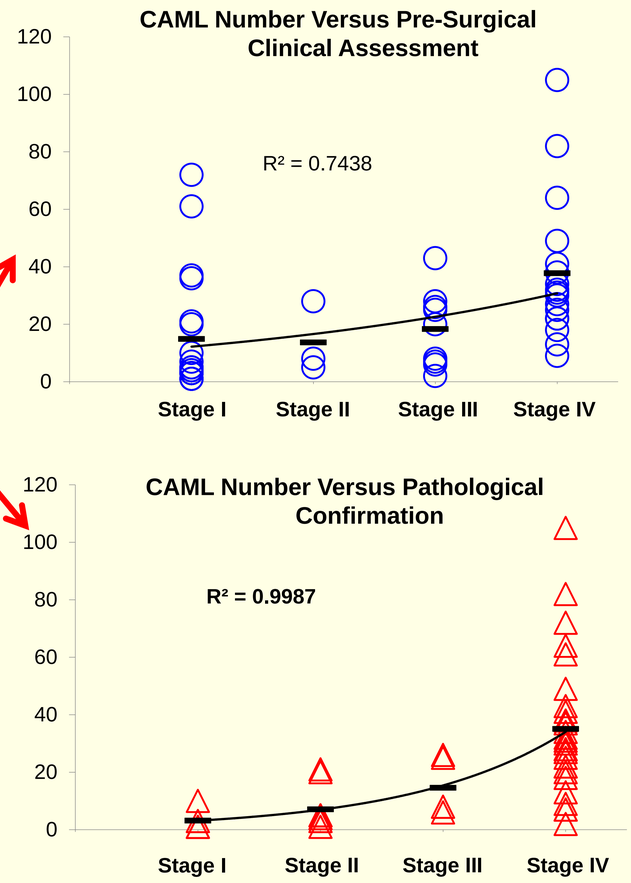


Figure 3. Comparing Number of CAMLs to type of therapy the patient is receiving and the relationship to staging

CONCLUSIONS

- CAMLs are circulating immune cells specific to malignant disease.
- The number of CAMLs appears to depend on therapy type.
- The number of CAMLs in patients undergoing chemotherapy is
  - weakly associated with pre-surgical clinical assessment and
  - highly correlative with pathological confirmation.
- These findings indicate that CAMLS may be a valuable supplement to current screening and staging procedures.

References

1. Adams DL, et al "Circulating giant macrophages as a potential biomarker of solid tumors." Pros Natl Acad Sci, 111(9):3514-3519, 2014
2. Adams DL, et al, "Cytometric Characterization of Circulating Tumor Cells Captured by Microfiltration and Their Correlation to the CellSearch® CTC Test." Cytometry 87(2): 137-144, 2015.
3. Cristofanilli M, et al, "Circulating tumor cells, disease progression, and survival in metastatic breast cancer." N Engl J Med 351:781-91, 2004
4. Plaks, V., Koopman, CD, & Werb, Z.,"Circulating Tumor Cells." Science 341(6151): 1186-1188, 2013.