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Clinical Significance of Circulating Cancer Associated Macrophage-Like Cells in Patients with Solid Tumors

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ABSTRACT

Blood-based biopsies can be used as a non-invasive method to recover a variety of cancer associated circulating cells, including Circulating Tumor Cells (CTCs) and circulating Cancer Associated Macrophage-like cells (CAMLs) from the blood of cancer patients. CAMLs are cancer specific giant polyploid cells circulating in the blood of patients with solid tumors. 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. Because of this, since their discovery, few studies have been done to investigate their clinical and biological significance in cancer. A long term prospective study of CAMLs in patients with solid tumors (n=147; breast, prostate, and lung) was undertaken to elucidate the clinical significance of CAMLs to overall survival .

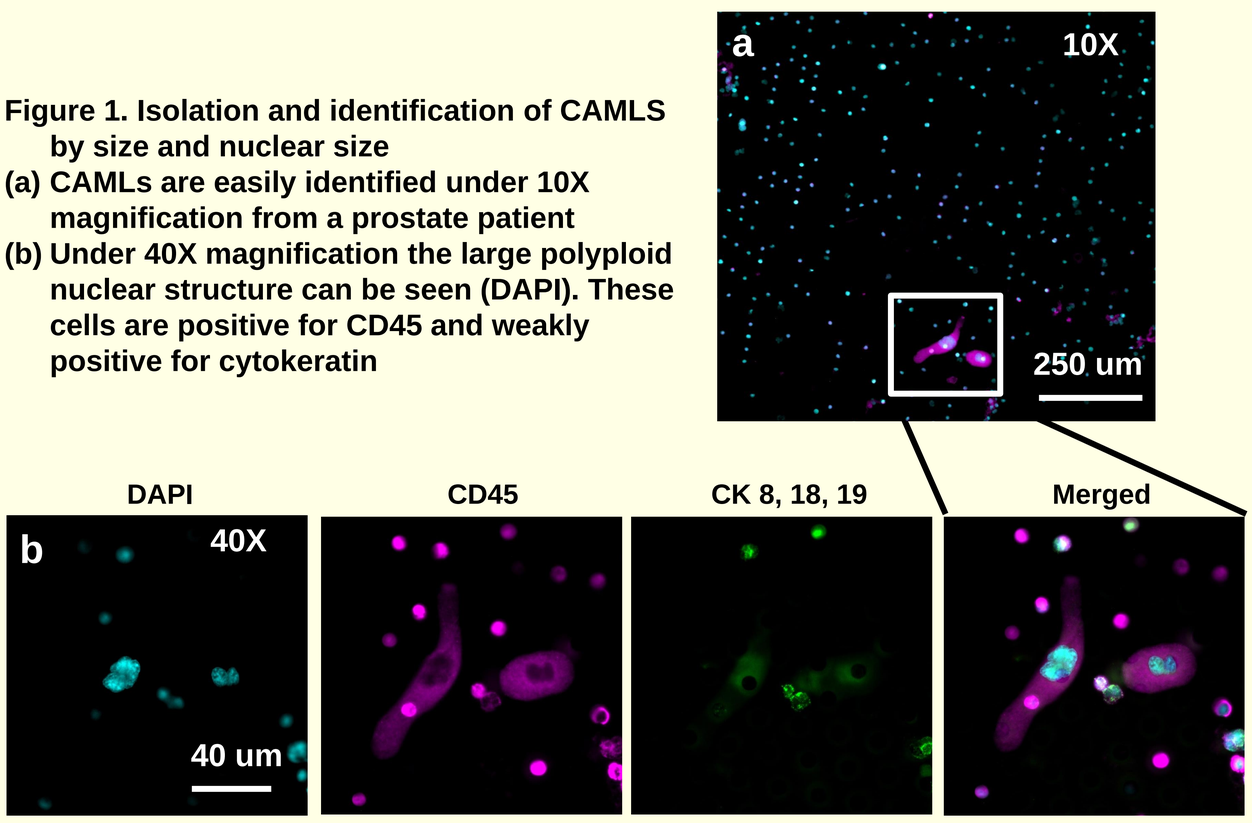

Figure 1. Isolation and identification of CAMLS by size and nuclear size
(a) CAMLs are easily identified under 10X magnification from a prostate patient
(b) Under 40X magnification the large polyploid nuclear structure can be seen (DAPI). These cells are positive for CD45 and weakly positive for cytokeratin

INTRODUCTION

CAMLs are specialized myeloid 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, though seen 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].

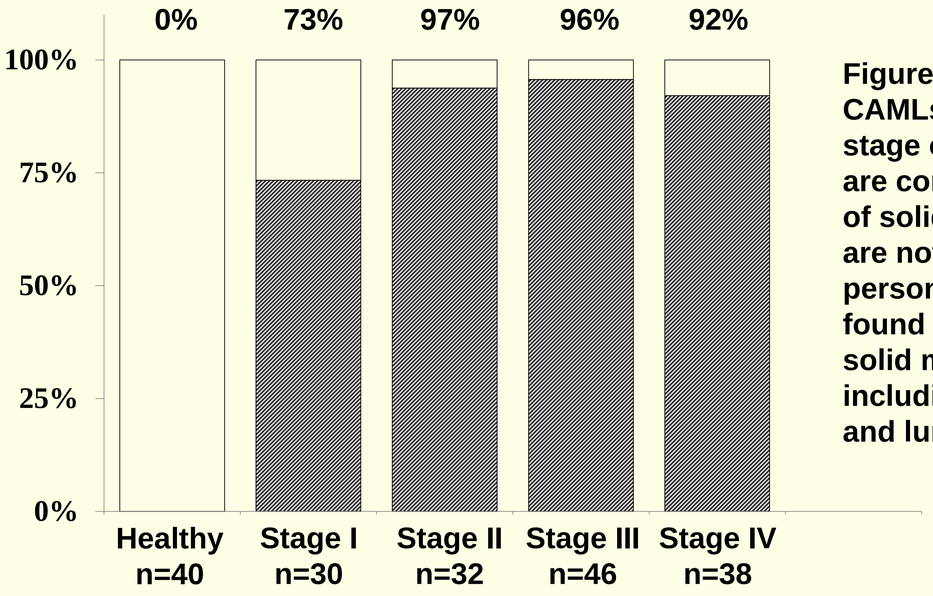

Figure 2. Presence of CAMLs in relation to stage of disease. CAMLs are common in all stage of solid malignancy but are not found in healthy persons. CAMLs are found in multiple types of solid malignancy including breast, prostate and lung cancer.

MATERIALS & METHODS

This multi-institutional study used peripheral blood samples from 147 cancer patients (stage I-IV) from breast (n=58), lung (n=58), and prostate (n=30). Blood was processed by the CellSieve™ microfiltration technique and stained for cytokeratin 8, 18 & 19, EpCAM, and CD45. CAMLs were enumerated and identified as large multinucleated circulating myeloid cells (Adams et al. PNAS 2014). We report CAML number compared with patient's stage and overall survival after 24 months. CellSieve™ microfilters were used to isolate CTCs and CAMLs from 7.5 mL of whole peripheral blood. The 7 µm pore size of CellSieve™ is capable of isolating both CTCs and CAMLs based on size. Collected cells were fixed, permeabilized, and stained with DAPI and antibodies against cytokeratin 8, 18 and 19, EpCAM, and CD45. CAMLs were defined as enlarged, multinuclear cells with *diffuse* cytoplasmic cytokeratin staining; they can be CD45+ or CD45-. CTCs were defined as *filamentous* cytokeratin cells that are CD45-.

RESULTS

- CAMLs were found in 90% patients with confirmed malignant disease (n=132/147)
  - CAMLs were found in 73% of stage I, 97% of stage II, 96% of Stage III, and 92% of Stage IV patients, regardless of cancer type (Fig. 2).
  - CAMLs were found in 93% of prostate, 91% of lung, and 93% of breast patient samples.
- Neither CAMLs nor CTCs, were found in any healthy individuals (n=40).
- Of the 86 patients remained on study for 2 years
  - 29 patients had ≥5 CAMLs/7.5mL and 35% (10 of 29) survived 24 months
  - 57 patients had <5 CAMLs/7.5mL, and 68% (39 of 57) survived 24 months
  - Hazard ration was 2.5 (CI95% 1.5-6.2) p=0.004

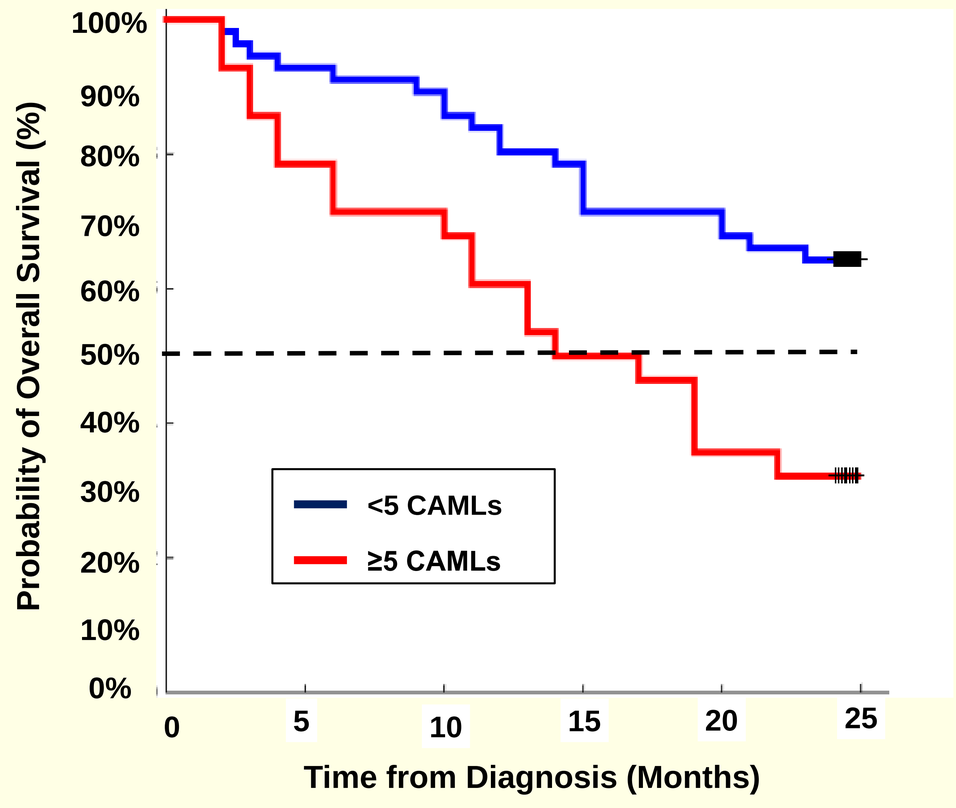

Figure 4. Kaplan Meier log rank analysis comparing patients with ≥5 CAMLs per sample (n=29) or <5 CAMLs (n=57). Hazard ratio=2.5 (CI95% 1.5-6.4) p=0.004.

CONCLUSIONS

- CAMLs can be used as a non-invasive blood based biopsy, to detect the anatomical presence of solid malignancies.
- Morphological identification of CAMLs is straightforward by their extreme size and large nuclear profile.
- CAMLs are commonly found in all stages of malignancy.
- CAML number appears as a prognostic valuable as assessed by overall survival over a 24 month period.

References

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