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Enlarged Stromal Macrophages found in cancer patient blood are associated with more aggressive disease and poorer prognosis in localized stage I/II solid tumors

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ABSTRACT

Using peripheral blood allows for the recovery of various cancer associated circulating stromal cells, including a previously described phagocytic macrophage defined as a Cancer Associated Macrophage-Like cell (CAML). CAMLs are phagocytic myeloid cells that derive from an immunological response to tumor presence and emanate from primary tumors. These phagocytic events by CAMLs can be easily quantified by CAML engorgement which has been described by numerous groups as an indicator of poorer clinical outcomes in a variety of cancer types. Using a filtration platform we analyzed the peripheral blood of untreated newly diagnosed breast, prostate and lung cancer patients (n=107) for CAMLs to determined their sensitivity and clinical prediction in localized disease

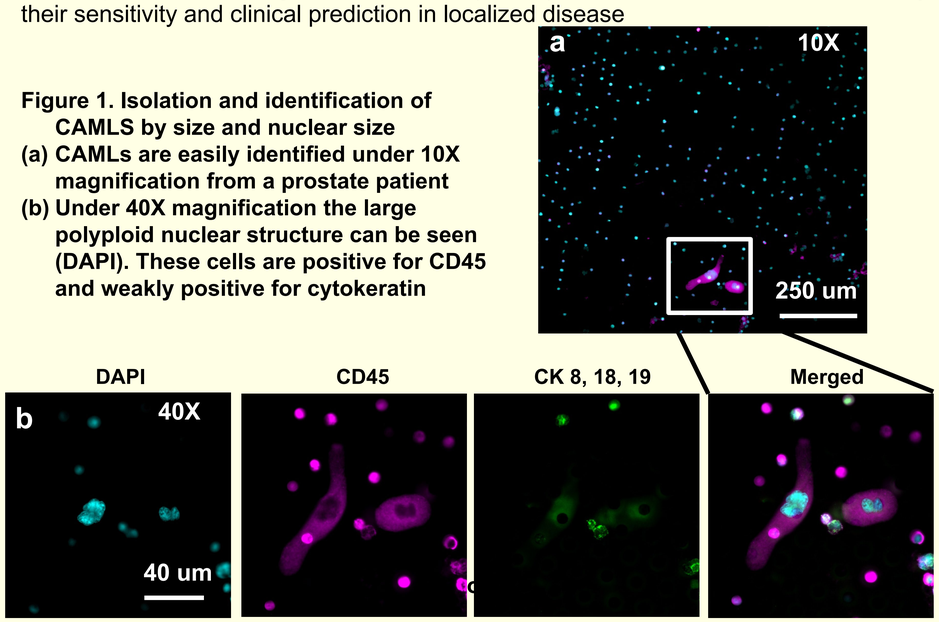

Figure 1. Isolation and identification of CAMLS by size and nuclear size
(a) CAMLs are easily identified under 10X magnification from a prostate patient
(b) Under 40X magnification the large polyploid nuclear structure can be seen (DAPI). These cells are positive for CD45 and weakly positive for cytokeratin

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[1-4]. However, while CAMLs are easy to identify by their large size and polyploid nucleus (**Figure 1**), 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 surface marker expression. CellSieve™ microfilters are size exclusion membranes that efficiently isolate CAMLs from whole blood, making it possible to study CAMLs in conjunction with and in relation to malignant disease.

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MATERIALS & METHODS

Three single blind multi-institutional prospective studies were undertaken using anonymized peripheral blood with informed consent under approved IRBs. Blood was taken from 107 cancer patients after confirmation of localized solid malignancy [stage I (n=43) or stage II (n=64)] all with pathologically confirmed breast (n=25), lung (n=40), or prostate cancer (n=42) (Table 1). CAMLs were isolated from whole peripheral blood by the CellSieve™ microfiltration technique and defined as enlarged, multinuclear cells with cytokeratin and/or CD45/CD14. Presence of enlarged CAMLs was then used to evaluate relapse-free survival (RFS) and overall survival (OS) hazard ratios (HRs) by censored univariate analysis at 5 years.

Table 1. Demographic Table of Patients

Demographic	Total Patients (n=107)
Age (Years): Median [Range]	65 [32-83]
Male	65 (61%)
Female	42 (39%)
Pathological Stage	
I	43 (40%)
II	64 (60%)
T	
1	47 (44%)
2	55 (52%)
3	4 (4%)
N	
0	76 (74%)
1 (or 1/2)	23 (22%)
unknown	8 (8%)
Cancer Types	
Breast	24 (21%)
Prostate	43 (40%)
Lung	42 (39%)
Grade	
1	7 (7%)
2	26 (24%)
3	32 (30%)
Unknown	42 (39%)
CAML Positivity # of Patients	89 (83%)
Average/7.5mL blood [Median]	2.5 [2]

RESULTS

- CAMLs were found in 83% of all cancer patients regardless of stage, averaging 2.5 CAMLs/7.5mL blood (**Figure 1**).
- CAMLs were found in 72% of Stage I and 91% Stage II disease.
- 30 patients relapsed, and 24 died within 5 years.
- Within 5 years, patients with ≥50um CAMLs had significantly worse clinical outcomes (Figure 2 and Figure 3).
- Specifically, patients with ≥50um CAMLs had
  - Worse RFS (HR=9.6, p<0.00001) and
  - Worse OS (HR=6.8, p=0.00054)

Figure 2. Kaplan-Meier PFS based on CAML Size at Diagnosis

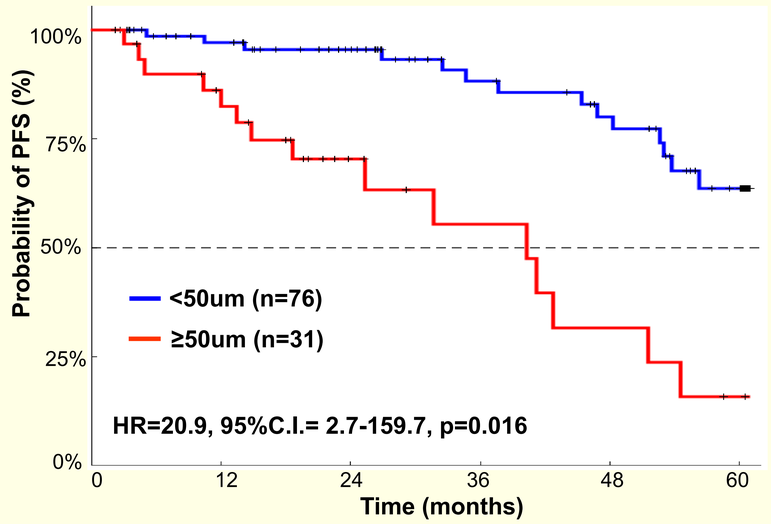
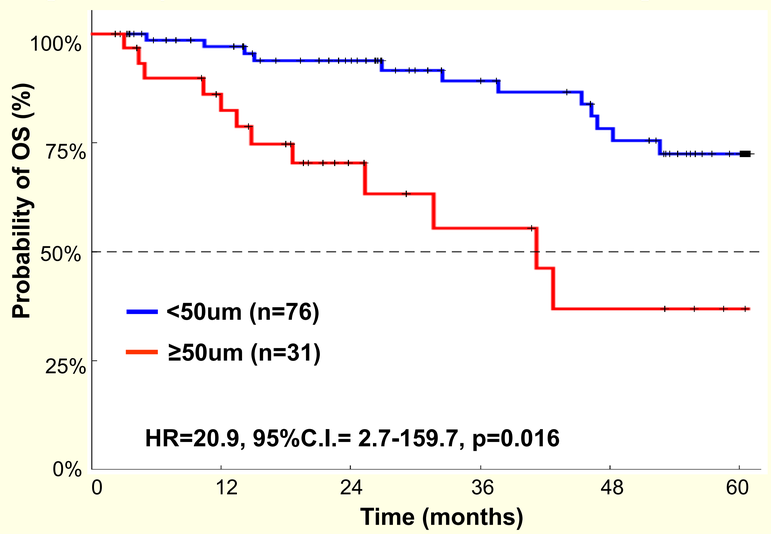


Figure 3. Kaplan-Meier OS based on CAML Size at Diagnosis



CONCLUSIONS

- Using data from three prospective clinical studies, we found that CAMLs, a circulating stromal cell subtype, are a sensitive blood based biomarker specific to patients with local neoplasm.
- CAMLs ≥50um found in blood at diagnosis are prognostic for highly aggressive tumors likely to relapse within 5 years.
- Larger studies evaluating each disease subtype are needed to validate these findings.

References

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