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CAMLs are a circulating stromal cell subpopulation that accurately predicts resistance and progression in treatment naïve lung cancer patients receiving definitive radiotherapy

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ABSTRACT

Cancer Associated Macrophage-Like cells (CAMLs) are a recently described circulating stromal cell common to the peripheral blood of cancer patients hypothesized to be a mechanism in cancer pathogenesis. We have previously described that treatment naive patients with circulating CAMLs ≥50μm is a significant independent prognostic indicator of progression free survival (PFS) in a variety of cancers. However, the clinical value of CAMLs in specific diseased cohorts as it relates to predicting response to treatment has not been evaluated. We present the results of a prospective study on treatment naïve lung cancer patients before induction, and if possible directly after completion, of definitive radiotherapy to determine if CAMLs are predictive of cancer progression within 2 years.

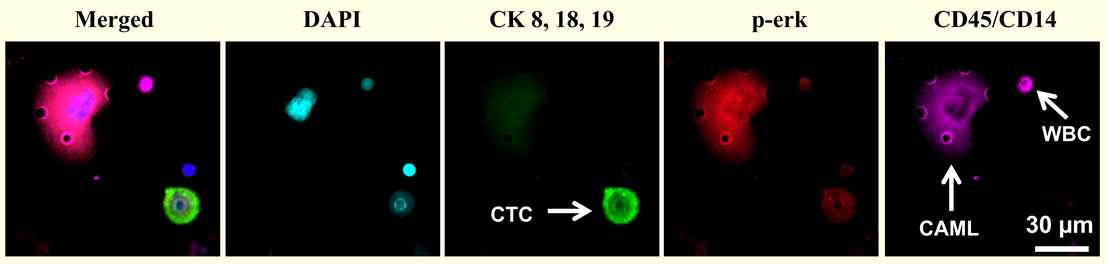


Figure 1. Example of CTC isolated with a CAML in a breast cancer patient. CTCs are Cytokeratin positive (green) and CD45/CD14 negative. In contrast, CAMLs are CD45/CD14 positive (purple) and may be weakly positive for Cytokeratin (green). White blood cells (WBCs) are normal sized CD45/CD14 positive cells.

INTRODUCTION

CAMLs are specialized myeloid polyploid cells transiting the circulation of patients in various types of solid malignancies and appearing in all stages of cancer[1-4]. While CAMLs are easy to identify by their large size and polyploid nucleus, they appear to present as stem cell like phenotype with multiple heterogeneous epithelial, myeloid, and endothelial markers (**Figure 1**). Size exclusion is the only known technique for isolating large cells from peripheral patient blood irrespective of their surface markers. CellSieve™ microfilters are size exclusion membranes which efficiently isolate CAMLs and circulating tumor cells (CTCs) from whole blood, making it possible to study both cell types in relation to malignant disease[1-4].

MATERIALS & METHODS

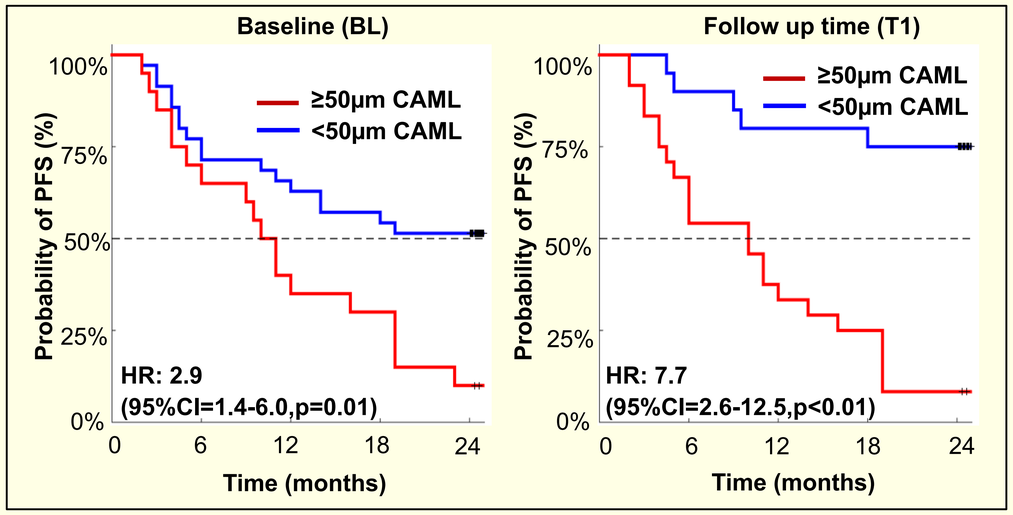
A 2 year single blind prospective study was undertaken, testing the relationship of enlarged CAMLs (≥50μm) to PFS of lung cancer patients before & after induction of definitive radiation therapy. To achieve a 2-tailed 95% power (α=0.05) we recruited a training set of 55 patients, all with pathologically confirmed lung cancer: Stage I (n=13), Stage II (n=7), Stage IIIa (n=10), Stage IIIb (n=18) & Stage IV (n=7).

Baseline (BL) blood samples were taken prior to start of therapy. If possible, a 2nd blood samples (T1) was taken after completion of radiotherapy (~60 days), n=46 patients. Blood was filtered by CellSieve™ filtration and CAMLs quantified. Analysis by CAML size of <50 μm or ≥50 μm was used to evaluate PFS hazard ratios (HRs) by censored univariate & multivariate analysis.

RESULTS

- CAMLs were found in 93% of BL samples averaging 3.2 CAMLs/7.5mL,
- At BL, patients with CAMLs of ≥50 μm had reduced PFS (HR=2.9) (**Figure 2**)
- At T1 patients with CAMLs of ≥50 μm had further reduced PFS (HR=7.7) (**Figure 2**)
- At BL, 90% of patients with a ≥50 μm CAML progressed in 2 years vs 46% of patients with a <50 μm CAML. (**Figure 2**)
- At T1, enlarged CAMLs were more accurate at predicting progression, with 92% of ≥50 μm CAML patients progressing vs 21% of patients with <50 μm CAMLs. (**Figure 3**)
- 100% of patients that had ≥50 μm CAMLs at both BL and T1 progressed. (**Figure 3**)
- Only 11% of patients with <50 μm CAMLs at both BL and T1 progressed. (**Figure 3**)
- CAML size was the most significant indicator of PFS and OS, independent of all other clinical variables

Figure 2. Analysis of PFS at BL vs FU based on size of CAMLs



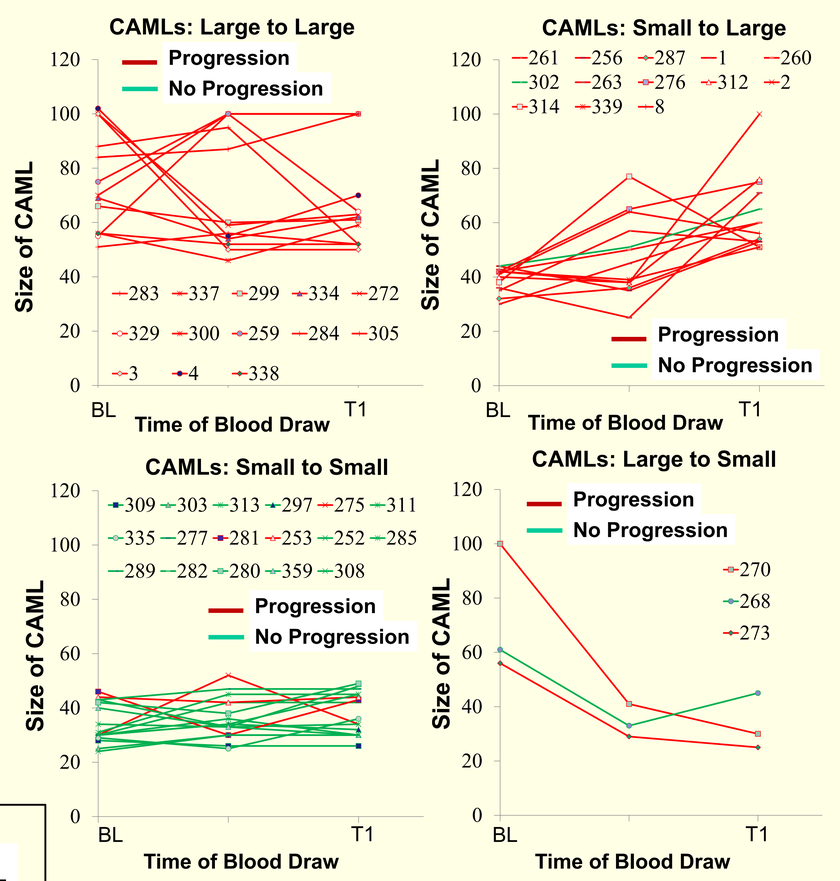


Figure 3. Changes of CAML size compared with progression or response between BL samples before starting treatment versus a T1 sample taken after completion of chemo radiation therapy in unresectable NSCLC

CONCLUSIONS

- Giant CAML sizes were prognostic both at pretreatment baseline as well as any change in size that occurred during and/or after therapy
- Giant CAMLs could represent a population of tumor stroma cells that may promote tumor progression
- Monitoring the presence of giant CAMLs and their sizes through the course of radiation therapy may predict cancer progression or death
- Prospective validation of giant CAMLs as a blood-based biomarker for risk stratification is ongoing through a R43/SBIR grant, results pending.

References

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