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Training and validation study for sequential monitoring of CAMLs in circulation to predict ongoing progression in lung cancer patients undergoing definitive radiotherapy

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ABSTRACT

Cancer Associated Macrophage-Like cells (CAMLs) are a recently described circulating stromal cell common in the peripheral blood of cancer patients whose presence is prognostic for progressive disease. Further, it has been shown that changes in CAML size (i.e. enlargement to greater than 50µm) might be predictive for poorer progression free survival (PFS) in a number of thoracic cancers, including lung cancer. We prospectively enrolled 104 unresectable non-small cell lung cancer (NSCLC) patients, with an initial training set review of 54 patients, to determine if change in CAML size after radiation therapy was predictive of PFS within 2 years.

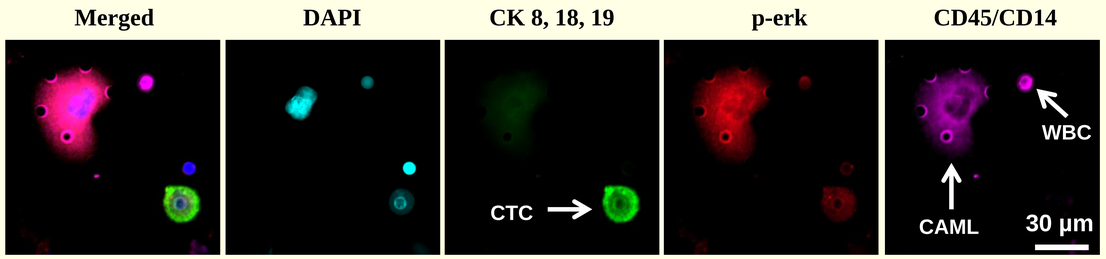


Figure 1. Example of CTC isolated with a CAML in a breast cancer patient. CTCs are Cytokeratin positive (green) and CD45/CD14 negative. In contrast, CAMLs are CD45/CD14 positive (purple) and may be weakly positive for Cytokeratin (green). White blood cells (WBCs) are normal sized CD45/CD14 positive cells.

INTRODUCTION

CAMLs are specialized myeloid polyploid cells transiting the circulation of patients in various types of solid malignancies and are common in all stages of cancer[1-3]. 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[1-3].

MATERIALS & METHODS

A 2 year single blind prospective study was undertaken to test the relationship of ≥50µm CAMLs to PFS based on imaging in lung patients before and after induction of chemoradiation, or radiation therapy. To achieve a 2-tailed 90% power (α=0.05) we recruited a training set of 54 patients and validation set of 50 patients all with pathologically confirmed unresectable NSCLC: Stage I (n=14), Stage II (n=16), Stage III (n=61) & Stage IV (n=13).

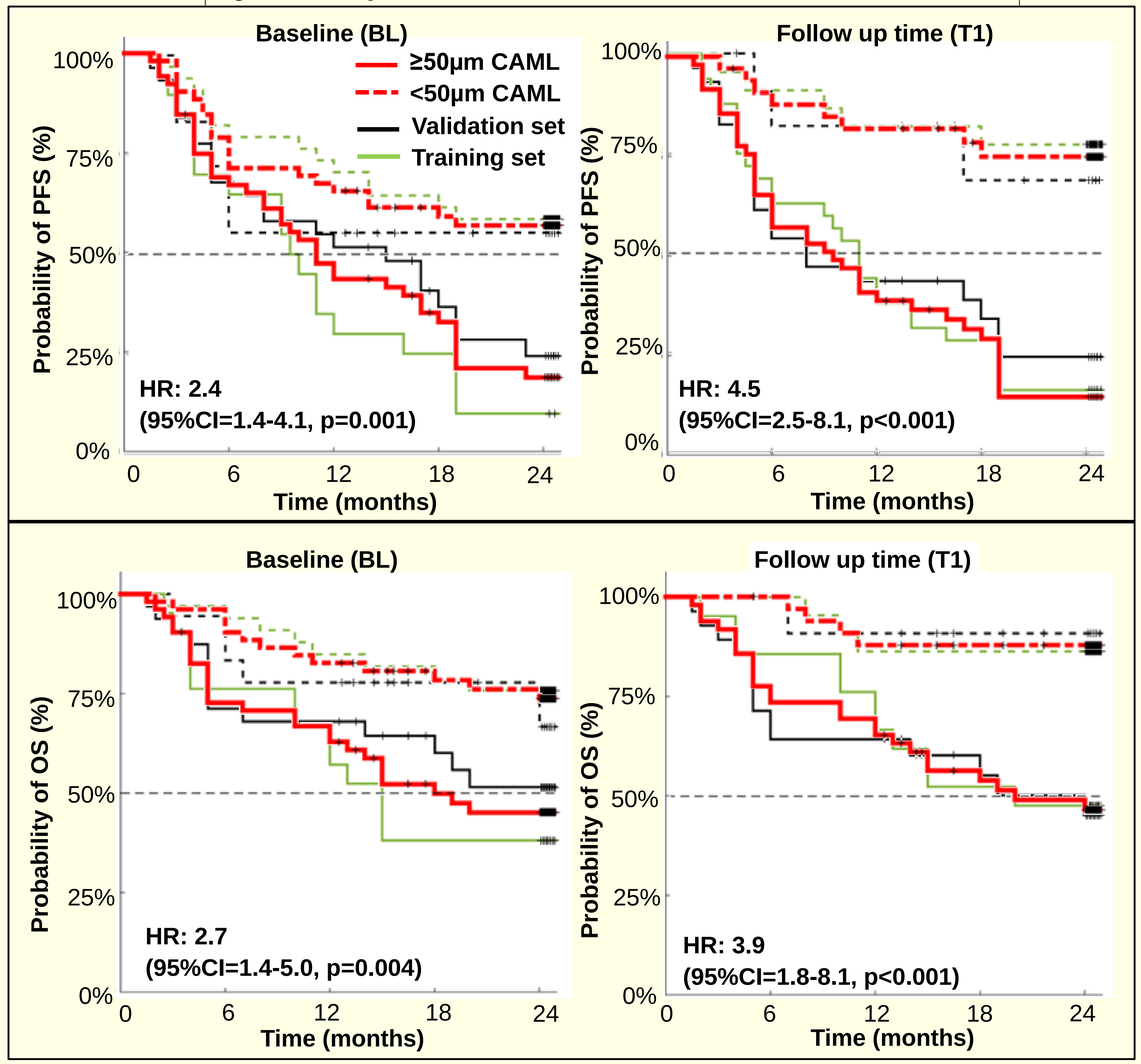
Baseline (BL) blood samples were taken prior to start of therapy and a 2nd blood sample (T1) was taken after completion of radiotherapy (~30 days). Blood was filtered by CellSieve™ filtration and CAMLs quantified. Analysis by CAML size of <49 µm or ≥50 µm was used to evaluate PFS hazard ratios (HRs) by censored univariate & multivariate analysis. .

RESULTS

- CAMLs were found in 95% of samples averaging 2.7 CAMLs/7.5mL sample at BL
- At BL, patients with CAMLs ≥50 µm had reduced PFS (HR=2.4) (**Figure 2**)
- At T1, 18 patients had increased CAML size ≥50 µm with reduced PFS (HR=4.5) (**Figure 2**).
- 76% of patients with ≥50 µm CAMLs at BL progressed within 24 months
- 83% of patients with ≥50 µm CAMLs at T1 progressed within 24 months
- CAML size was the most significant indicator of PFS and OS, independent of all other clinical variables (**Table 1**)

Variable	PFS (p value)	OS (p value)
CAML Size at T1 (<50µm vs ≥50µm)	**<0.001**	**0.004**
Stage	0.531	0.452
Tumor Size (T1, T2, T3 or T4)	0.187	**0.035**
Node neg, local, distant	0.511	0.162
Metastatic pos vs neg	**0.032**	0.319
Grade	0.365	0.322
Histology	0.928	0.426
Concurrent Chemotherapy	0.701	0.708
Radiation Modality	0.384	0.382
Radiation Fraction	0.566	0.299
Total Radiation Dose	0.590	0.844
M/F	0.814	0.973
Age	0.578	**0.028**

Figure 2. Analysis of PFS and OS at BL vs T1 based on size of CAMLs



CONCLUSIONS

- In unresectable NSCLC patients, enlargement of CAMLs within 30 days of treatment induction is an indicator of progression.
- We trialed and validated that a single ≥50 µm CAML, after completion of radiotherapy, is a significant independent indicator of poorer prognosis.
- Changes in CAML size during therapy may indicate the efficacy of therapy and could potentially help shape subsequent therapeutic decisions
- Further prospective validation of giant CAMLs as a blood-based biomarker for risk stratification is ongoing through a R43/SBIR grant, results pending.

Funding Sources

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