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# Sequential Monitoring of Circulating Stromal Cells from Blood is Predictive of Progression in NSCLC Patients Undergoing Anti-PD-L1 Therapy after Definitive Chemoradiation Therapy

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## ABSTRACT

Cancer Associated Macrophage-Like cells (CAMLs) are a recently described circulating stromal cell common in the peripheral blood of patients with solid tumors. We have previously described that in non-small cell lung carcinoma (NSCLC), patients with CAMLs ≥50µm after completion of chemo-radiation therapy (CRT) have been shown to have worse progression free survival (PFS) and overall survival (OS). However, with the recent addition of anti-PD-L1/PD-1 therapies in conjunction with CRT as standard of care, it has yet to be evaluated whether CAMLs remain predictive for monitoring progression in NSCLC patients post anti-PD-L1/PD-1 therapy.

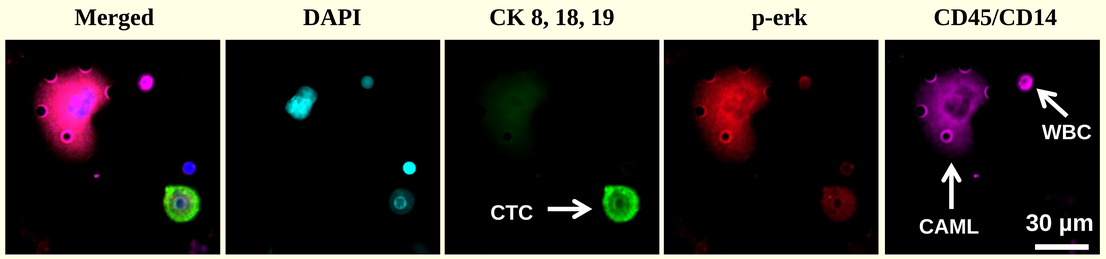


**Figure 1. Example of CTC isolated with a CAML in a breast cancer patient. CTCs are Cytokeratin positive (green) and CD45/CD14 negative. In contrast, CAMLs are CD45/CD14 positive (purple) and may be weakly positive for Cytokeratin (green). White blood cells (WBCs) are normal sized CD45/CD14 positive 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 phenotypes with multiple heterogeneous epithelial, myeloid, and endothelial markers (**Figure 1**). Size exclusion is the only known technique for isolating CAMLs from peripheral patient blood irrespective of their surface markers, making it possible to study CAMLs in 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 CRT and anti-PD-L1/PD-1 therapy. We recruited 104 patients with pathologically confirmed unresectable NSCLC Stage II (n=14), Stage III (n=83), Stage IV (n=3), and locally recurrent disease (n=4). If possible, Baseline (BL) blood samples were taken prior to start of therapy (n=96). A 2nd time point blood sample (T1) was taken at the end of radiotherapy (~40 days) (n=95). A 3rd time point blood sample (T2) was taken after induction of anti-PD-L1/PD-1 therapy (e.g. Durvalumab, Pembrolizumab, etc.), average 3.5 months post BL (n=80). Blood was filtered by CellSieve™ filtration and CAMLs were quantified. Analysis by CAML size of <50 µm or ≥50 µm was used to evaluate PFS and OS hazard ratios (HRs) by censored univariate & multivariate analysis at each available time point.

## References

1. Adams DL, et al “Circulating giant macrophages as a potential biomarker of solid tumors.” Proc Natl Acad Sci, 111(9):3514-3519. 2014
2. Cristofanilli M, “Liquid Biopsies in Solid Tumors” *Springer Intl Pub.* 2017
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## RESULTS

- CAMLs were found in 87% of samples averaging 2.9 CAMLs/7.5mL sample
- At BL, patients with CAMLs ≥50 µm had similar PFS (HR=1.1) and OS (HR=1.4) (**Figure 2**).
- At T1, post-CRT but before consolidated immunotherapy, CAMLs ≥50 µm were associated with reduced PFS (HR=3.2) and OS (HR=2.9) (**Figure 2**).
- Post-CRT immunotherapy was given at median 3.2 months (range 0-12 months).
- At T2, after 1 cycle of immunotherapy, CAMLs ≥50µm continued to be associated with reduced PFS (HR=2.8) and OS (HR=3.3) (**Figure 2**).
- CAML size was a significant early predictor of PFS and OS, independent of all other clinical variables

## CONCLUSIONS

- In 104 patients, a single ≥50 µm CAML, after either completion of CRT or anti-PD-L1/PD-1 therapy, was a significant predictor of poorer prognosis.
- CAML size at T1 (Post-CRT & pre-anti-PD-L1/PD-1) stratified patients who appeared to respond to CRT treatment.
- Changes in CAML size after CRT therapy may indicate the efficacy of consolidated immunotherapy 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.
- CAML size after completion of any treatment cycle (CRT or immunotherapy) appeared to predict for benefit of that treatment.

## Funding Sources

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## Figure 2. Analysis of PFS and OS at BL, T1, and T2 based on size of CAMLs