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Sequential Monitoring of PD-L1 on Circulating Stromal Cells Predicts Outcomes in Localized Unresectable Stage II NSCLC Treated with Immunotherapy after Definitive Chemoradiation

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ABSTRACT

Previously we have reported on a type of tumor derived circulating stromal cell found in the blood of cancer patients (pts), described as phagocytic giant macrophages, which appear to represent the inflammatory state of the tumor microenvironment and have been defined as a circulating Cancer Associated Macrophage-Like cells (CAMLs). In non-small cell lung carcinoma (NSCLC), CAMLs can express PD-L1, which can dynamically change and be monitored throughout chemoradiation therapy (CRT). However, it is not known if PD-L1 on CAMLs has a relationship to immunotherapy (IMT) efficacy after CRT in local NSCLC. We studied a pilot group of Stage II unresectable NSCLC patients treated with (n=20) or without (n=20) IMT agents after CRT to evaluate CAML PD-L1 expression as it relates to progression free survival (PFS) & overall survival (OS).

MATERIALS & METHODS

A multi-year single blind prospective study was undertaken to test the relationship of PD-L1 expression in CAMLs, for NSCLC pts before and after induction of CRT. Patients were treated either with standard of care CRT with consolidation Durvalumab (n=14) or under a clinical trial treated with concurrent CRT with consolidation Atezolizumab (n=6). A control group of patients treated with CRT alone, absent of IMT (n=20), were evaluated during the same period (n=13 before 2018). In all, we recruited 40 pts with pathologically confirmed unresectable stage II NSCLC prior to CRT. Anonymized blood samples (15mL) were taken ~1 month after completion of CRT with written informed consent and with local IRB approval. Blood was filtered by CellSieve™ filtration, and CAMLs quantified for PD-L1 expression using a binary score (0/1=low or 2/3=high) (**Figure 2**), to evaluate PFS & OS hazard ratios (HRs) by censored univariate analysis at 24 months.

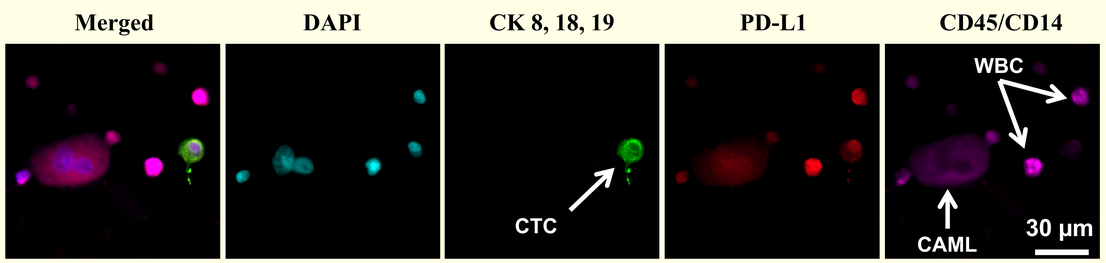


Figure 1. Example of CTC isolated with a CAML in a cancer patient. CTCs are Cytokeratin positive (green) and CD45/CD14 negative. CAMLs are CD45/CD14 positive (purple) and may be weakly positive for Cytokeratin (green). White blood cells (WBCs) are normal sized CD45/CD14 positive cells.

INTRODUCTION

CAMLs are specialized myeloid polyploid cells transiting the circulation of patients in various types of solid malignancies and common to all stages of cancer[1-3]. CAMLs are easy to identify by their large size and polyploid nuclei, that appear to present as phagocytic cells with multiple heterogeneous immune phenotypes (**Figure 1**). Size exclusion is the only known technique for isolating large cells from peripheral patient blood irrespective of surface markers. CellSieve™ microfilters are size exclusion membranes that 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].

RESULTS

- CAMLs were found in 83% of all samples (average=3.8 CAMLs/15mL)
- PD-L1 expression was similar in both cohorts (**Figure 2**)
- Tumor PD-L1 expression analysis was not possible, as only n=11 tumor biopsies were available.
- After completion of CRT:
  - In patients treated with CRT alone. CAMLs PDL1 was not prognostic for PFS (HR=1.8, p=0.696) or OS (HR=2.2, p=0.581) (**Figure 3**)
  - In patients treated with IMT. High CAML PD-L1 patients trended non-significantly for improved PFS (HR=5.9 p=0.079) and OS (HR=16.7, p=0.087) (**Figure 3**)

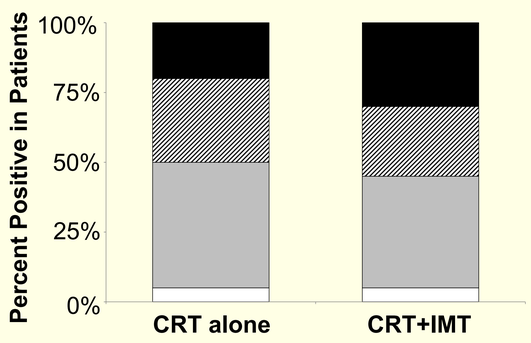
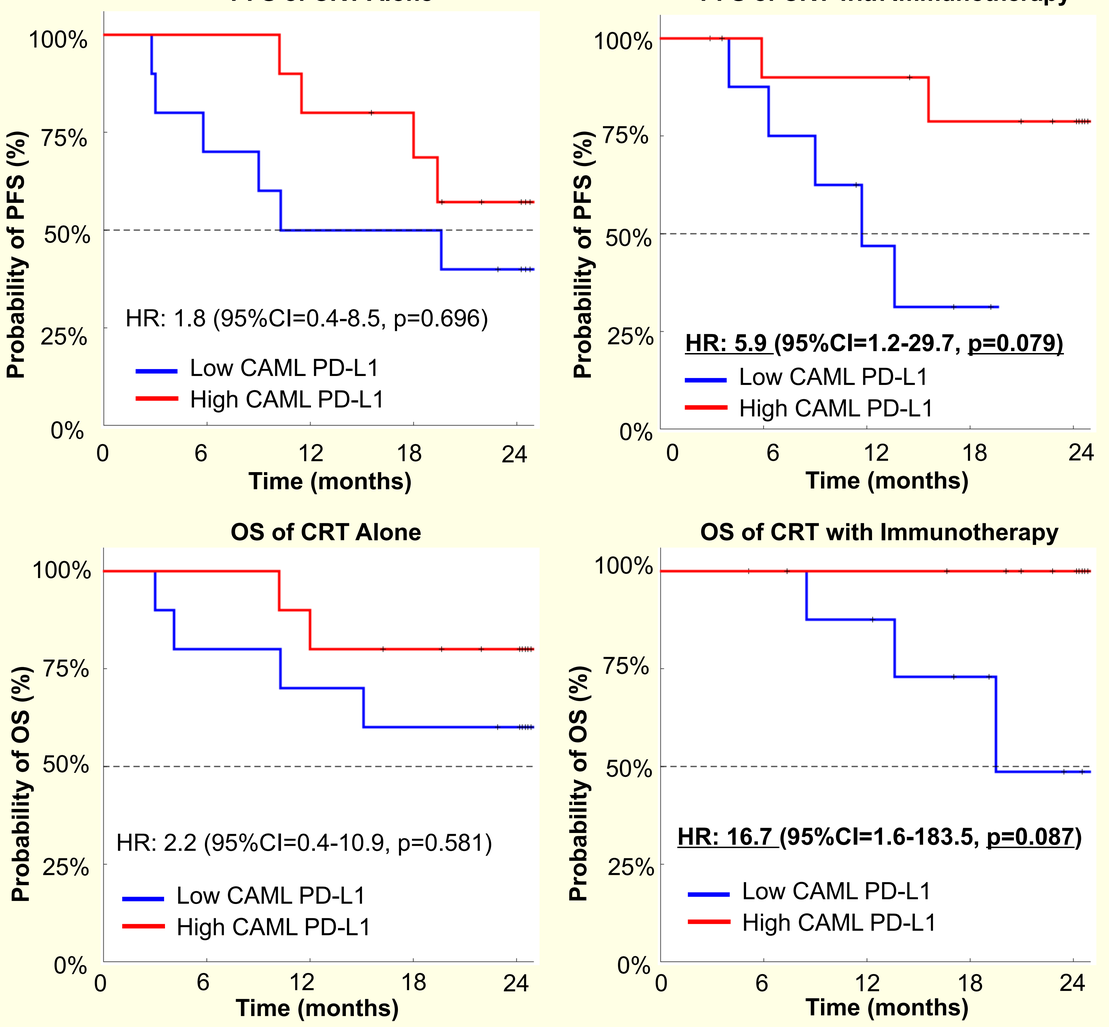


Figure 2. PD-L1 expression distribution was similar in patients treated with CRT alone cohort versus patients treated with CRT with IMT cohort. White boxes=No cell PD-L1 measurement available. Grey boxes=low PD-L1. Black/White strip=Medium expression. Black=high expression.

Figure 3. Analysis of PFS and OS based on PD-L1 expression in CAMLs After CRT



CONCLUSIONS

- Tissue PD-L1 expression has limited use in IMT treatment decisions, because of poor correlation with clinical response.
- Monitoring of PD-L1 expression in circulating stromal cells in blood may predict for response to IMT.
- In this study, the data suggests that in unresectable stage II NSCLC, high PD-L1 expression on CAMLs after CRT completion may identify patients that are more responsive to PD-L1 immunotherapies.
- Prospective studies are ongoing through a R43/SBIR grant which may validate these results and increase the significance of these findings.

References

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