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Expression of PD-L1 on Circulating Stromal Cells Predicts Immunotherapy Response in Unresectable Non-Small Cell Lung Carcinoma After Definitive Chemoradiotherapy

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ABSTRACT

Circulating stromal cells (CStCs) have been found to be common in the peripheral blood of cancer patients and hypothesized to be a blood based biomarker for monitoring cancer treatment. It has previously been described that the dynamic changes of PD-L1 expression during chemoradiotherapy (CRT) could be tracked using circulating stromal cells. However, how these changes relate to PD-L1/PD-1 immunotherapy (IMT) response is unstudied. We prospectively monitored PD-L1 expression in 2 cell types found in circulation (Circulating Tumor Cells [CTCs] and Cancer Associated Macrophage-like Cells [CAMLs]) in locally advanced non-small cell lung cancer (NSCLC) patients (pts) treated with Atezolizumab (Atezo) after definitive CRT (n=39) or in pts with CRT alone (n=40).

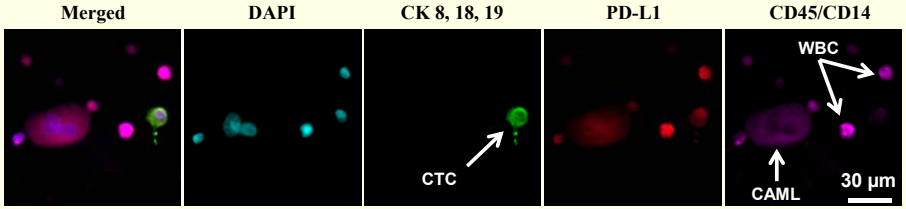

Figure 1. Example of CTC isolated with a CAML in a cancer patient. CTCs are Cytokeratin positive (green) and CD45/CD14 negative. CAMLs are CD45/CD14 positive (purple) and may be weakly positive for Cytokeratin (green). White blood cells (WBCs) are normal sized CD45/CD14 positive cells.

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 nucleus, 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 their surface markers. CellSieve™ microfilters are size exclusion membranes which efficiently isolate CAMLs and 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 in pts with locally advanced NSCLC in 40 patients treated with CRT alone, and from a phase II DETERRED trial (NCT02525757) where Atezolizumab was added for 1 year after completing CRT (n=10), or concurrently and after CRT (n=30). Samples from 39 of 40 pts from the DETERRED study were available for analysis. Baseline blood samples (7.5 ml) were drawn prior to start of CRT (T0), and a second sample was drawn ~1 month after completing CRT (T1), but prior to induction of Atezo for 10 patients. Blood was processed by CellSieve™ microfilters; stained for cytokeratin/PDL1/CD45 to identify CTCs and CAMLs. PD-L1 intensity was measured and grouped by 2 scores: 0/1-low expressing and 2/3 high expressing. PD-L1 levels from circulating cells were used to evaluate PFS and OS. Significance was assessed by log-rank testing.

References

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RESULTS

- CAMLs were found in 91% of T0 & 94% of T1 samples.
- CTCs were found in 26% of T0 & 33% of T1 samples.
- In the 40 patients that received only CRT:
  - At T0, PD-L1 in CStCs was high in 20% of pts.
  - At T1, PD-L1 in CStCs was high in 56% of pts.
  - PD-L1 expression did not correlate to PFS or OS **(Figure 2)**.
- In 39 pts that received CRT with Atezolizumab
  - At T0, PD-L1 in CStCs was high in 45% of pts
  - PD-L1 expression at T0 did not correlate to PFS or OS (**Figure 2**).
  - At T1, PD-L1 in CStCs was high in 45% of pts
  - At T1, pts with high PD-L1 had significantly better response to Atezolizumab PFS (HR 3.9, p=0.027), and OS (HR 12.1, p=0.001)

CONCLUSIONS

- Tissue PD-L1 expression not used in IMT treatment decisions due to limited correlation with clinical responses
- Sequential blood monitoring of PD-L1 expression in circulating stromal cells in blood may predict for response to IMT.
- These data suggests that CRT altered PD-L1 expression, and monitoring dynamic changes of PD-L1 in CStCs may predict immunotherapy effectiveness in NSCLC after CRT.
- Further prospective validation of CStCs as a blood-based biomarker for risk stratification in IMT is ongoing through a R43/SBIR grant, results pending.

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Figure 2. Analysis of PFS and OS at T0 vs T1 based on size of CAMLs