PD-L1 Expression on Immune Cells from Solid Tumor Patients: Implications for Tumor Immunity and Immunotherapy

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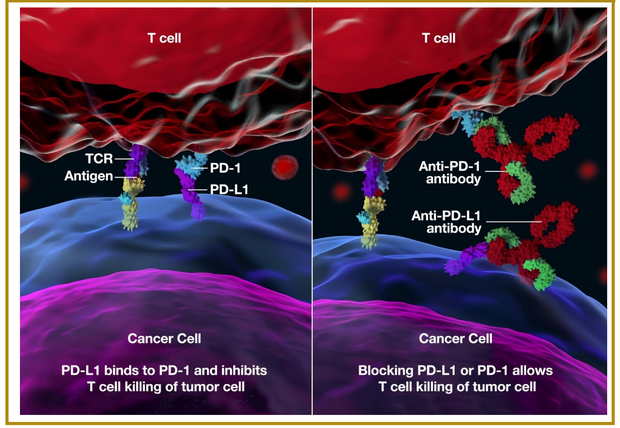
Introduction

Programmed death-ligand 1 (PD-L1) is a key immune checkpoint molecule that regulates immune responses by binding to PD-1 on T cells, contributing to immune tolerance and suppression. PD-L1 expression in solid tumors has become an important biomarker for evaluating the efficacy of immune checkpoint inhibitors in cancer therapy. Characterization of PD-L1 expression on immune cells, particularly CD8+ T cells, in patients with various solid tumors, including melanoma, non-small cell lung cancer (NSCLC), and breast cancer was performed using cryopreserved peripheral blood mononuclear (PBMC) samples using flow cytometry. In addition to PD-L1, T cell activation, proliferation (e.g., CD69, Ki67) and exhaustion (e.g., PD-1) were analyzed to gain a comprehensive understanding of immune cell status. PD-L1 expression was highly variable across patients within solid tumor indications, with elevated expression found on CD8+ T cells, suggesting an upregulation in response to persistent tumor antigenic stimulation. Furthermore, PD-L1+ CD8+ T cells often expressed markers of exhaustion, such as PD-1, indicating a potential role for PD-L1 in T cell dysfunction and immune evasion.

PD-1/PD-L1 Immune Checkpoint Blockade



Multi-Parameter Flow Method

Whole blood was collected at clinical sites and centralized PBMC isolation using SepMate™ tubes ficoll density separation. PBMCs were cryopreserved and stored in the vapor phase of LN2 to maintain viability. Longitudinal PBMC samples from each subject were batch tested together. PBMCs were thawed into complete medium and cells counted for viability by automated cell counting with AO/PI with a Nexcelom Cellometer.

An immunophenotyping panel with markers to identify immune cell subsets, activation and exhaustion markers was used to stain the PBMC. Markers included CD45, CD3, CD4, CD8, CD14, CD16, CD56, CD11c, CD123, CD69, Ki67, PD-1, and PD-L1. Antibodies were purchased from BioLegend, BD Biosciences or eBioscience. Assay controls included single color compensation controls and a healthy donor PBMC inter-assay control with full panel stains and fluorescence minus one or more markers (FMO/FMX) for objective setting of analysis gates.

Cells were stained with a Live/Dead dye (Life Technologies) prior to the addition of Fc block. Cells were centrifuged, washed with staining buffer and stained with surface markers. After centrifugation, cells were resuspended in fixation/permeabilization buffer (eBioscience) and then washed in the permeabilization buffer. The cell pellet was resuspended and stained with intracellular markers. Cells were washed and resuspended in staining buffer prior to acquisition with a BD LSRFortessa™ X-20 flow cytometer using FACSDiva software. Data was analyzed with FlowJo.

Summary of Results

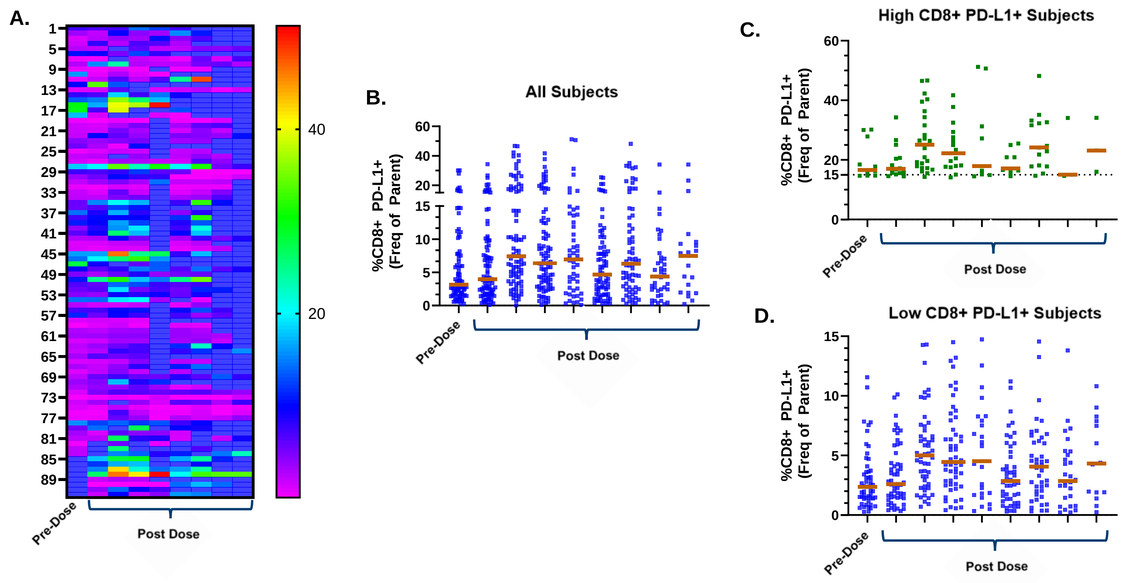


Figure 1. PD-L1 Expression on CD8+ T cells

In Figure 1A., Heatmap representing expression of %CD8+ PD-L1+ T cells in 91 solid tumor patients from pre-dose through eight timepoints from Cycle 1 through Cycle 3. (B.) % CD8 PD-L1+ expression on cryopreserved PBMC for all subjects from pre-dose through Cycle 1 (D1, D5, D7, and D14), Cycle 2 (D1, D7), and Cycle 3 (D1, D7). CD8+ PD-L1 expression in these subjects ranged from low (<15%) PD-L1 expression to higher expression (≥15%), dependent on the timepoint. (C.) Higher % CD8+ PD-L1+ expression was observed in 40% of the patients, (37 out of 91), where expression was ≥ 15%, (ranging from 15-50%) in at least one or more timepoints over the course of the study. (D.) Lower % CD8+ PD-L1+ expression was observed in 59% of the patients, (54 out of 91), where expression was <15% and greater variability of PD-L1 expression was observed.

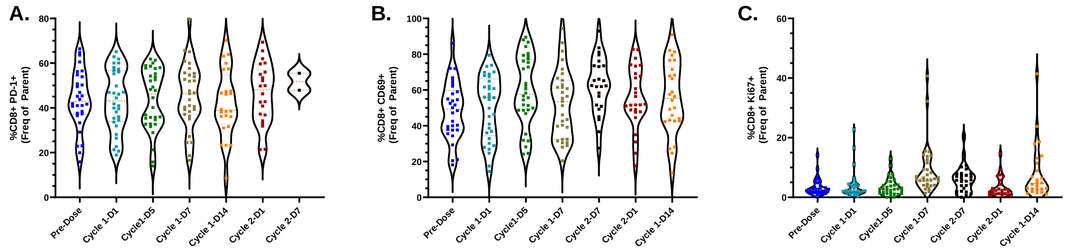
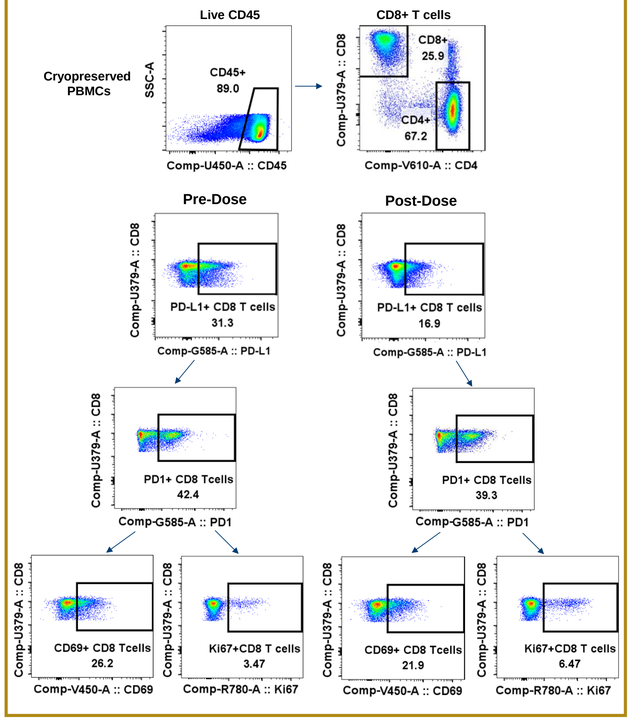


Figure 2. Expression of PD-1, CD69, and Ki67 in CD8+ T cells

In Figure 2A-2C., In the 34 subjects with higher % CD8+PD-L1+ T cell expression (>15%), in at least one or more timepoints over the course of the study, the expression of PD-1, CD69 and Ki67 was evaluated on CD8 T cells. (A.) Expression of exhaustion marker PD-1 on % CD8+ PD-1+ T cells, ranged from 8-70%, average 44% ±13%. (B.) Expression of activation marker CD69 on %CD8+ CD69+ T cells ranged from 13-92%, average 55% ± 18%. (C.) Proliferation, evaluated by Ki67 on %CD8+ Ki67+ T cells ranged from 1-41%, average 5.6% ± 5.3%.

Summary of Results

Figure 3. High PD-L1+ subject is illustrated below, with Pre-Dose and Post Dose Cycle 1 Day 1 to demonstrate the expression of PD-L1, PD-1, CD69 and Ki67 on CD8 T cells for this subject.



Conclusion

We observed that 42% of patients had high expression of PD-L1 on CD8+ T cells at some point during the study. The elevated expression of PD-L1 trended towards occurring early in the study suggesting an upregulation of PD-L1 in response to persistent tumor antigenic stimulation rather than due to high levels of T cell proliferation as low levels of Ki67 were observed at early timepoints.

These findings underscore the complexity of PD-L1 expression by immune cells and its role in shaping immune responses in solid tumor patients. Through integrating PD-L1 profiling with other markers of immune activation and exhaustion, this highlights the potential of PD-L1 as a predictive biomarker for immunotherapy response and provides insights into the mechanisms of immune evasion in solid tumor cancers.

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