# PD-L1 expression in NSCLC MDSCs and its potential use as a biomarker to determine treatment response through dimensionality reduction of flow cytometric data

**Roser Salvia[1], Laura G. Rico[1], Jolene A. Bradford[2], Michael D. Ward[2], Marc Sorigué[4], Teresa Morán[3], Joan Climent[1], Rafael Rosell[3,4], and Jordi Petriz[1]**

[1] Institut d'Investigació Germans Trias i Pujol, ICO-Hospital Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badalona, Barcelona, Spain; [2] Thermo Fisher Scientific, Eugene, Oregon, USA; [3] Institut Català d'Oncologia, Hospital Germans Trias i Pujol (HGTiP), Badalona, Barcelona, Spain; [4] Quirón Dexeus University Hospital, Dr Rosell Oncology Institute, Barcelona, Spain

## INTRODUCTION

Immunotherapy is currently a major treatment option for cancer. Non-small cell lung cancer (NSCLC) treatment options include 5 approved immunotherapies to target the Programmed Cell Death Protein 1/Programmed Cell Death Protein Ligand 1 (PD-1/PD-L1) checkpoint with the aim of avoiding tumor immune escape. Accurate detection of PD-L1 is crucial to calculate the Tumor Proportion Score and decide the line of treatment. Therefore, we developed a minimal sample perturbation methodology to target PD-L1 by flow cytometry (Rico et al., 2021). This protocol allows the identification of conformational changes in circulating Myeloid-Derived Suppressor Cells (MDSCs). In this study, we present prospective evaluation of PD-L1 expression in MDSCs from patients with NSCLC undergoing anti-PD-L1/PD-1 immunotherapy.

**Table 1. Clinical treatment strategy and immunotherapy administered according to patients' status.**

| Treatment line | | Immunotherapy (IT) |
|---|---|---|
| First line (>50% PD-L1) | IT | Pembrolizumab |
| First line (<50% PD-L1) | Chemotherapy + IT | Pembrolizumab / Nivolumab |
| Second line | IT | Pembrolizumab / Nivolumab / Atezolizumab |
| Consolidation | Chemotherapy + Radiotherapy + IT | Durvalumab |

## MATERIALS AND METHODS

Peripheral blood (PB) samples from NSCLC patients (n=40) were collected in EDTA-anticoagulated tubes prior and during anti-PD-L1/PD-1 immunotherapy, which consisted of pembrolizumab ($\alpha$-PD-1), nivolumab ($\alpha$-PD-1), atezolizumab ($\alpha$-PD-L1) or durvalumab ($\alpha$-PD-L1) administration (**Table 1**). PB samples were immediately processed using our previously reported minimal sample perturbation protocol and acquired on the Attune™ NxT Flow Cytometer (Thermo Fisher). MDSCs were stimulated with phorbol esters (PMA) for 5 minutes and were identified according to HLA-DR$^{-/lo}$CD33$^{+}$CD11b$^{+}$ immunophenotype (**Figure 1A**). Furthermore, MDSCs can be classified according to the CD33 and CD11b expression in monocytic MDSCs (m-MDSCs, CD33$^{hi}$CD11b$^{+}$) and polymorphonuclear MDSCs (PMN-MDSCs, CD33$^{+}$CD11b$^{hi}$). FCS files analysis was performed using FlowJo™ (v.10), and FCS files were concatenated, downsized, and analyzed with tSNE, UMAP and FlowSOM, based on their stimulation (PMA or DMSO) and clinical outcome classification (**Table 2**).

**Table 2. Clinical outcome of NSCLC patients.**

| Clinical Outcome | Patient Number |
|---|---|
| Response to treatment | 15 |
| Stabilization of disease | 8 |
| Progression of disease | 10 |
| Non-Assessable | 7 |

In "Response to treatment" group, tumor size is diminished; in "Stabilization of disease" group, tumor size is maintained; in "Progression of disease" group, tumor size is increased; and in the "Non-Assessable" group, patients died without further tests.

## RESULTS AND DISCUSSION

UMAP and tSNE allowed comprehensive data visualization of PD-L1 expression in NSCLC samples (**Figure 2**). Unstimulated MDSCs showed no PD-L1 reactivity (**Figure 1B**). After PMA stimulation, PD-L1 underwent conformational changes, making possible the reactivity with the anti PD-L1 monoclonal antibody (**Figure 1C**) and clustering separately when compared with unstimulated MDSCs (**Figure 2**). Moreover, the frequency of polymorphonuclear MDSCs was increased when compared with the monocytic fraction (**Figure 1C**).

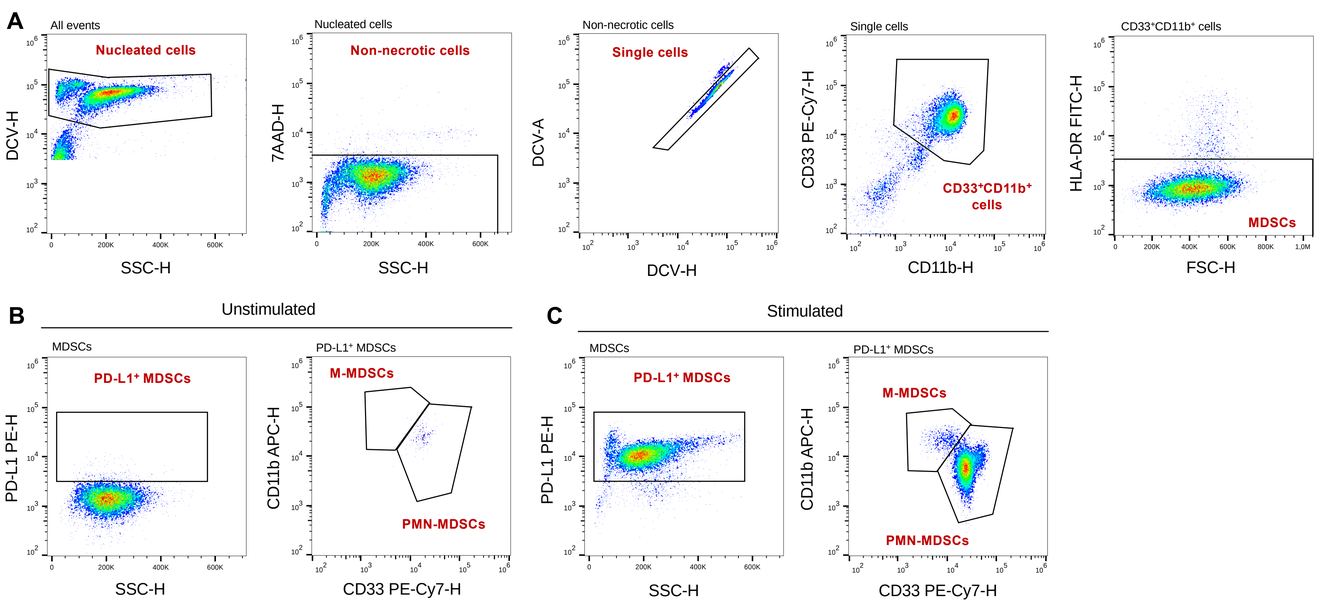


**Figure 1. Gating strategy for identification and classification of MDSCs.** Representative analysis of a peripheral blood sample from a NSCLC patient. **A)** Nucleated cells are selected based on DCV+ events, and necrotic cells and doublets are discarded. CD33$^{+}$ CD11b$^{+}$ HLA-DR$^{-/lo}$ events are then selected. **B)** PD-L1 is not reactive in non-stimulated cells, whereas in **(C)** PMA-stimulated cells are highly reactive to the anti-PD-L1 monoclonal antibody.

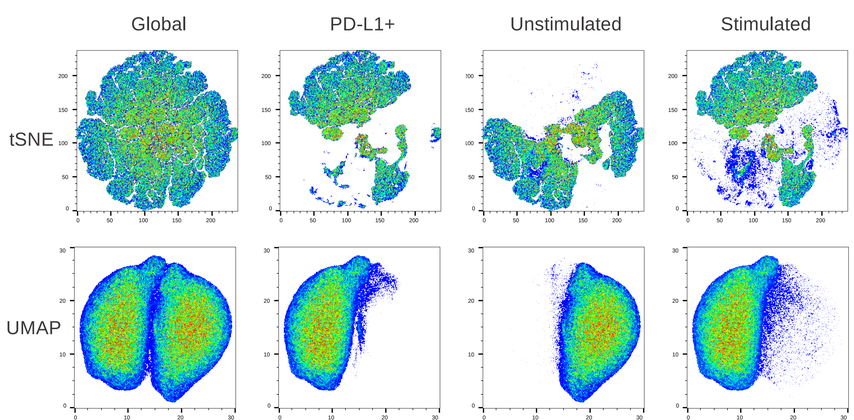


**Figure 2. t-SNE and UMAP displays used to demonstrate PD-L1+ reactivity.** t-SNE and UMAP analysis of n=40 NSCLC patients was performed in Myeloid-Derived Suppressor Cells, after selecting the following MDSC lineage markers: CD33, CD11b, HLA-DR and PD-L1.

FlowSOM provided self-organizing maps with a common backbone for group comparisons. Stimulated and unstimulated specimens displayed complementary FlowSOM organizing maps, whereas individual patients exhibited particular features. In stimulated samples, FlowSOM analysis of three main clinical outcome groups showed differences in polymorphonuclear MDSC population (**Figure 3**).

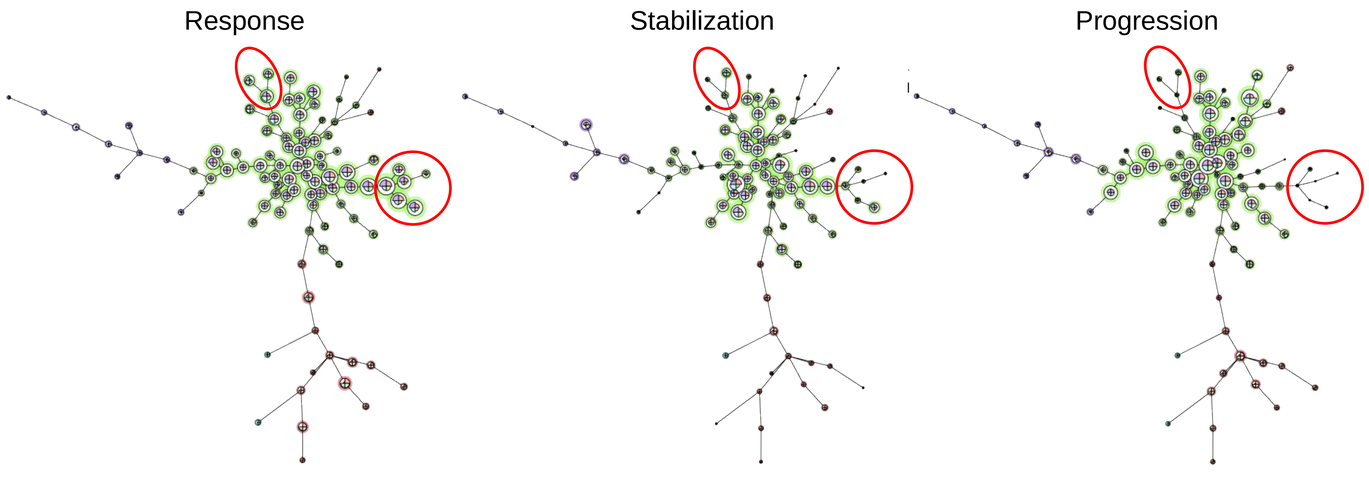


**Figure 3. FlowSOM self-organizing maps defining differential profiles related to the clinical status.** Response to treatment, stabilization and disease progression groups are displayed in three self-organizing maps. The green population are PMN-MDSCs whereas the red cluster contains m-MDSCs and the violet cluster contains both m-MDSCs and PMN-MDSCs. As encircled in red, differences between the three groups are mainly associated with PMN-MDSCs.

## CONCLUSIONS

Further analysis will be needed to ascertain how the conformational changes of PD-L1 may help to accurately predict immunotherapy efficacy. Moreover, the feasibility of determining PD-L1 expression in NSCLC MDSCs may have a potential use as a biomarker to determine treatment response through dimensionality reduction. In fact, data analysis can be beneficial to improve diagnosis as well as to predict immunotherapy response early in the course of treatment.

## ACKNOWLEDGEMENTS

IGTP Germans Trias i Pujol Research Institute · ThermoFisher SCIENTIFIC · ISAC INTERNATIONAL SOCIETY FOR ADVANCEMENT OF CYTOMETRY

* This work was partially done at the Josep Carreras Leukemia Research Institute (Barcelona, Spain)