Differential Expression of PD-L1 on Myeloid Derived Suppressor Cells as a potential screening approach for cancer immunotherapy in Multiple Myeloma

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INTRODUCTION

Multiple myeloma (MM) is characterized by the accumulation of malignant plasma cells in the bone marrow (BM), constituting a critical microenvironment for the survival, expansion and chemoresistance of myeloma cells. Although new therapies have markedly improved the results in the treatment of MM, today remains incurable, but manageable once diagnosed.

Programmed Death Ligand-1 (PD-L1) has been identified as responsible to suppress immune responses through the interaction with Programmed Death 1 (PD-1). In MM, PD-L1 is expressed in MM plasma cells, Myeloid Derived Suppressor Cells (MDSCs) and various non-hematopoietic cells. PD-1 is expressed in T-cells and it has been shown to negatively regulate T-cell receptor (TCR) signaling. The interaction between PD-L1 and PD-1 has been reported to decrease TCR-mediated proliferation and cytokine production. Thus, PD-L1 might play an important role in tumor immune evasion and drug resistance and is being considered as a therapeutic target for this disease.

The aim of this study was to design and evaluate a direct screening assay to identify MM MDSCs PD-L1+ cells using flow cytometry, for potential use as a point of care diagnostic for MM and other advanced human hematologic cancers. This new experimental approach may provide a useful supplementary approach to current immunohistochemical methods.

MATERIALS AND METHODS

A total of 60 cases diagnosed with MM were studied. Peripheral blood (PB) and/or BM samples were analyzed at diagnosis, follow-up, and relapse or pre-apheresis time points. Mobilized PB specimens from patients with MM were also analyzed. PD-L1 staining was combined with CD11b, CD33, and HLA-DR to detect MDSCs using no-lyse no-wash (NLNW) methods on the Attune™ NxT Flow Cytometer (Thermo Fisher). Phorbol 12-Myristate 13-Acetate (PMA) was used for 10 min at 37ºC to stimulate PD-L1 expression on MDSCs. Vybrant™ DyeCycle™ Violet Stain (Thermo Fisher) was used to discriminate nucleated cells from erythrocytes and debris. Violet side scatter was collected from the 405 nm laser with a 405/10 bandpass filter. For violet SSC, the accuracy of the height parameter is better preserved than for 488 nm blue SSC because the hemoglobin absorption of a coincident erythrocytes reduces its highest signal relative to the leukocytes.

RESULTS AND DISCUSSION I

We implemented a novel multicolor panel for the screening of PD-L1 expression in MM, consisting of 5-colors, 4-laser excitation, and no color compensation. Figure 1 shows a representative acquisition protocol with the gating strategy and representative dot plots to analyze PD-L1 expression on CD11b^{+}/CD33^{+}/HLA-DR$^{low/neg}$ MDSCs. Using 4 lasers (violet, red, yellow and blue) avoids color compensation and simplifies sample acquisition.

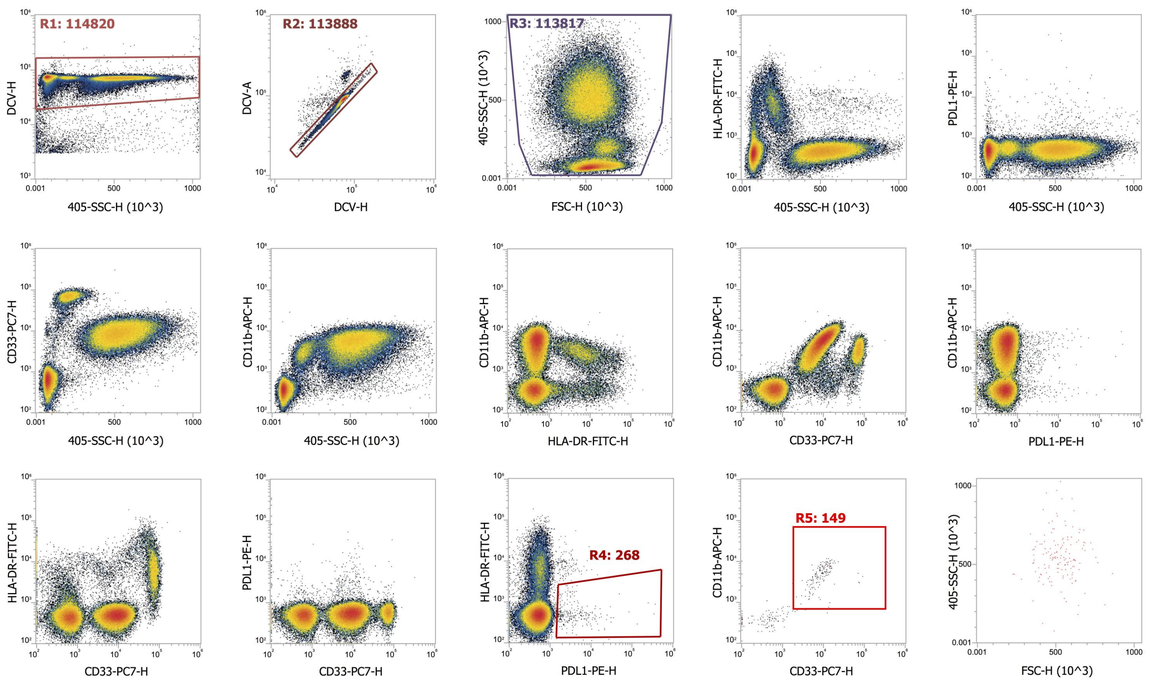

Figure 1. Representative acquisition protocol for the screening of PD-L1+ MDSCs. Gate **R1** was used to discriminate nucleated cells from erythrocytes and debris. **R2** was used to discriminate doublets using DNA staining. **R3** was finally used to eliminate apoptotic cells and debris. Dot-plots representing FITC-HLA-DR, APC-CD11b, PE-PD-L1, and PE-Cy7-CD33 versus SSC, and dual color dot-plots are displayed. PD-L1+ MDSCs were selected as HLA-DR$^{low/neg}$ /PD-L1^{+} in **R4**, and CD11b^{+}/CD33^{+} in **R5**. Samples were acquired on the Attune™ NxT Flow Cytometer (Thermo Fisher). Gating was sequential for all other plots. The statistics in the region represents count of the gate.

RESULTS AND DISCUSSION II

The persistent concern that sample preparation causes loss or alteration of specific fragile cells has also grown in the face of ever increasing scrutiny of smaller and smaller subdivisions of cell types. The possibility of assaying more than one functional cellular aspect in combination with phenotype, increases the potential for discovery of clinically relevant cell subsets. NLNW assays can be applied across a wide range of samples, and the work presented here focuses on PD-L1 expression on MDSCs. Reagent developments also present new opportunities for NLNW live cell analysis.

In this work, NLNW methods were used to study the alteration of PD-L1 expression on MDSCs from pre-clinical and clinical MM patients. We investigated the changes in PD-L1 expression after PMA treatment and found negative to positive conversion, from 1 to 418-fold increase after stimulation (Figure 3). Significant numbers of PD-L1+ MDSCs were also detected in leukapheresis products (data not shown).

Below, figure 2 shows two representative cases with different PD-L1 turnover after stimulation. Briefly, cells were stimulated for 10 min at 37ºC in the presence or absence of PMA. The upper row shows non-responding cells to a PMA stimulus. The expression of PD-L1 in DMSO (control) was restricted to 238 cells, and the number of cells with expression of PD-L1 was only increased to 516 cells with PMA. The lower row shows responding cells to a PMA stimulus. The expression of PD-L1 in DMSO (control) was restricted to 882 cells, and the number of cells with expression of PD-L1 was increased to 114,752 cells with PMA. The PD-L1+ population was calculated over the total number of acquired leukocytes. This different relative order of magnitude of PD-L1 expression after stimulation with PMA, showed important changes that cannot be detected without stimulation.

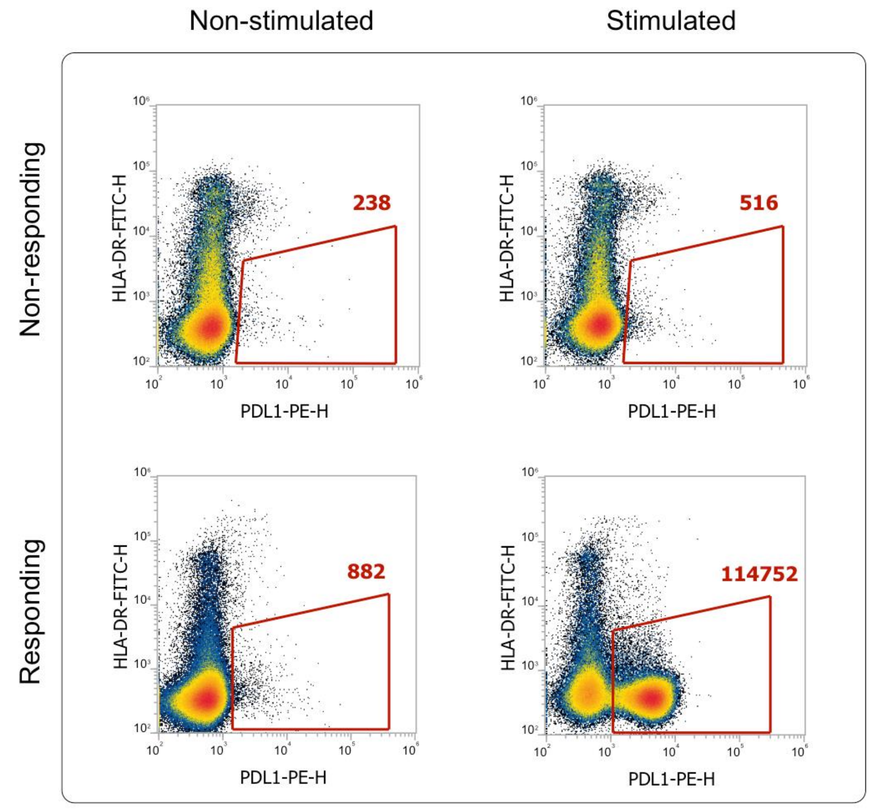

Figure 2. Representative cases with different PD-L1 turnover after stimulation. The upper row shows non-responding cells to a PMA stimulus. The lower row shows responding cells to a PMA stimulus. PD-L1+ cells are represented in terms of cell counts. Samples were acquired using Attune™ NxT Flow Cytometer (Thermo Fisher).

RESULTS AND DISCUSSION III

PD-L1 turnover after stimulation was performed in a total of 60 cases diagnosed with Multiple Myeloma. Figure 3 shows PD-L1 fold-increase of 23 MM patients bone marrow stimulated for 10 minutes at 37ºC with PMA. Fold-change was calculated as the ratio between stimulated PD-L1+ MDSCs and non-stimulated PD-L1+ cells. The PD-L1+ population was calculated over the total number of acquired leukocytes. Fold-change in PD-L1 between responding and non-responding groups appears to have an important regulatory factor that may relate to differences in underlying mechanisms within patients. This variability may help to explain the different response to targeted immunotherapy against PD-L1.

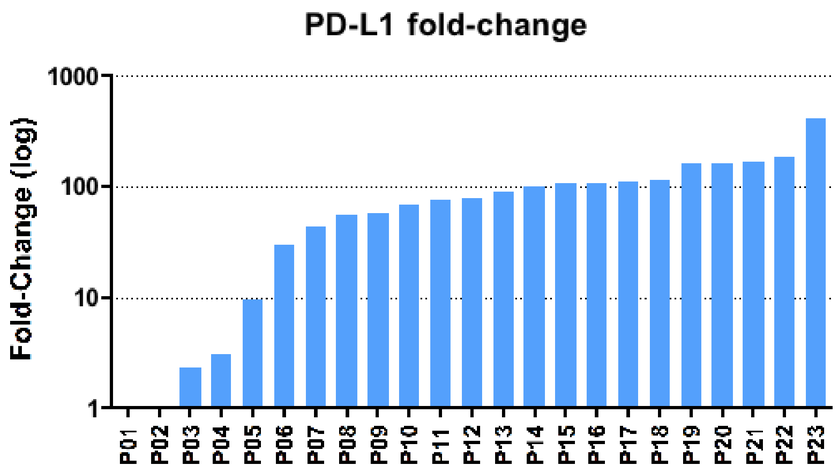

Figure 3. PD-L1 fold-change in a series of 23 multiple myeloma patients. Fold-change was calculated as the ratio between stimulated PD-L1+ and non-stimulated PD-L1+ MDSCs. The MDSC PD-L1+ population was calculated as the percentage of total leukocytes. Patients P01 to P23 showed a wide variability of fold-change (1 to 418).

CONCLUSIONS

Phenotypic characterization is not sufficient to identify MDSCs and an additional proof of the immunosuppressive function is needed. PD-L1 appears to be rapidly translocated to the cell surface in response to PMA stimulation. Although the mechanisms by which PD-L1 translocates to the cell surface is still unclear, these may be related to mechanisms that support tumor progression and therapeutic response of multiple myeloma. Important research must be done to elucidate the mechanism by which PD-L1 is translocated and the clinical relevance of those patients with higher PD-L1 fold-change. Future experiments will attempt to further dissect to what degree stimulation increases PD-L1 expression on MDSCs and how this information can be translated into benefit for immunotherapy.

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