Translational Oncology 14 (2021) 100969

Contents lists available at ScienceDirect

Translational Oncology

journal homepage: www.elsevier.com/locate/tranon

Letters to the Editor

Unmasking the expression of PD-L1 in Myeloid Derived Suppressor Cells: A case study in lung cancer to discover new drugs with specific on-target efficacy

PD-L1 expression on tumor cells and immune cells could predict response to immunotherapy [1]. However, tiny biopsy samples can result in false-negative PD-L1 evaluation [2]. Herein, we describe the accurate identification of PD-L1 on peripheral blood myeloid derived suppressor cells (MDSCs).

A 72-year-old male, never-smoker, was diagnosed with recurrent lung adenocarcinoma, PD-L1 negative (*22C3),* without actionable mutations. The patient was treated with nivolumab, and paclitaxel. After progression, he was re-treated with nivolumab plus glutaminase inhibitor until persistence of adrenal and distant lymph node metastasis was observed. Atezolizumab plus T cell immunoglobulin mucin-domain containing-3 (TIM3) inhibitor was administered as third line therapy. The patient completed 11 cycles. Afterwards, the left adrenal gland was irradiated and a muscle metastasis in the left gluteus was resected. Targeted Next Generation Sequencing (NGS) in metastatic tissue and blood were negative for actionable alterations. The patient received further treatment with a novel anti-PD-L1 (PDR001) combination. However, further progression was identified with widespread bone and lymph node metastases (More details in Supplementary Figure 1). Peripheral blood samples were serially collected before and after immunotherapy for the assessment of MDSCs and PD-L1 expression by multi-color flow cytometry.

Prior to immunotherapy (day 0), peripheral blood smear analysis showed mostly neutrophils and monocytes, and lymphocytes were clearly reduced (Supplementary Fig. 2A and B). Hematology analysis showed a decreased number of lymphocytes (5.5%), and a marked increase in monocytes (13%) and neutrophils (80.2%). At initial assessment, red-cell count, hemoglobin and hematocrit showed decreased concentrations. White-cell analysis by multi-color flow cytometry showed that PD-L1 was dramatically increased after Phorbol 12-myristate 13-acetate (PMA) stimulation. Peripheral blood cells were mostly PD-L1+ MDSCs (81.50% polymorphonuclear PMN-MDSCs and 7.74% monocytic M-MDSCs) (Fig. 1B).

Surprisingly, non-stimulated cells expressed undetectable levels of PD-L1, as shown by the flow cytometry method when DMSO was used (Fig. 1A). This suggests that PD-L1 displays some variation of spatial conformation [3] in response to PMA stimulation, which may confer a critical enhancement in binding affinity. This variability may help to explain the different response to targeted immunotherapy against PD-L1, also suggesting that phenotypic characterization is not sufficient to identify MDSCs and an additional proof of the immunosuppressive function is needed. PD-L1 was studied in a total of 5 samples, before and after immunotherapy (day 0, 1, 4, 6 and 8) showing differences in PD-L1 expression (Supplemental Fig. 3 and Supplemental Figure 4A) as well as in the relative number of MDSCs (Supplemental Figure 4B). Merging all together into a single FCS file using a process called concatenation, peripheral blood samples were effectively compared (Fig. 1C and D). Dimensionality reduction was performed on the concatenated data set. After PMA stimulation, PD-L1+ cells were displayed based on the expression of 6 markers using t-SNE (t-distributed stochastic neighbor embedding) visualization. The axes show dimensionless values that were assigned to individual cells by the t-SNE algorithm, which places PD-L1+ MDSCs that have similar expression profiles close to one another. Eliminating MDSCs by promoting PD-L1 stabilized unfolded states on both PMN- and M-MDSCs could improve immunotherapy efficacy. High frequency of PMN-MDSCs [4] is an adverse prognostic factor in lung [5] and head and neck cancer [6,7]. This conformational change may be associated with a PD-L1 immunoregulatory mechanism that affects therapies targeting the PD-1/PD-L1 checkpoint. Conformational changes, accumulation, expansion, and survival of MDSCs require research for PD-L1 unfolding. Monitoring peripheral blood MDSCs and identification of drugs with PMA-like effects is highly warranted. In view of these conformational properties, analysis of accumulation, expansion and survival of pathological immunosuppressive MDSCs could help to better understand and overcome the mechanisms of immunotherapy resistance, by developing new treatment strategies aimed at promoting PD-L1 stabilized unfolded states. Altogether, our findings may indicate the clinical relevance of peripheral blood MDSCs assessment and immunosuppressive pattern, as well as the need to find compounds that by themselves exhibit a PMA-like effect aimed at abrogating MDSCs. Limitations of this study include the enrollment of only one patient and a short follow–up period, because exitus occurred after a few days of analysis. Important research must be done to elucidate the mechanism by which PD-L1 is translocated and the clinical relevance to 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. The feasibility of determining PD-L1 expression on MDSCs and its potential use as a biomarker to determine treatment response is promoting new ways to study cancer, particularly in the promising area of immunotherapy.

https://doi.org/10.1016/j.tranon.2020.100969

Received 29 July 2020; Received in revised form 13 November 2020; Accepted 20 November 2020

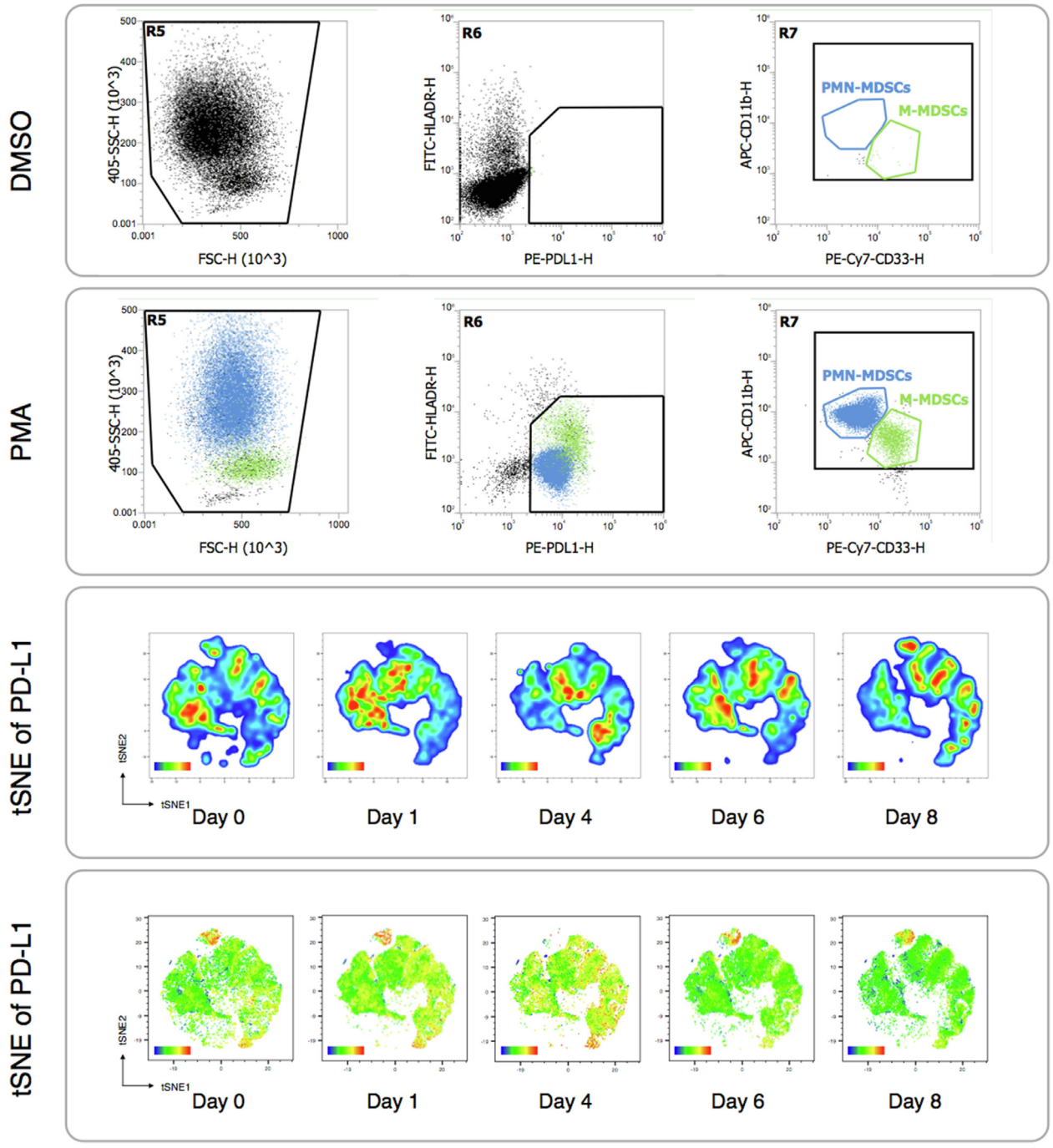

Fig. 1. Evaluation of PD-L1 expression and clustering analysis in terms of cell number and antigenic density comparisons across immunotherapy.
Evaluation of PD-L1 fold-increase of a 72-year-old male, a lifetime non-smoker, diagnosed with lung adenocarcinoma in 2014 the day before immunotherapy administration (day 0) and day 1, 4, 6 and 8 before immunotherapy. Panel A and B show a representative peripheral blood analysis on day 0stimulated for 5 min at 37 °C with PMA. Numbers in regions R5 to R7 indicate gated cell counts. Fold-change was calculated as the ratio between stimulated PD-L1+ MDSCs and non-stimulated PD-L1+ cells. PMN-MDSCs were identified by their expression of PD-L1 together with $CD33^{+}CD11b^{high}HLA\text{-}DR^{-/low}$ and M-MDSCs were identified by their expression of PD-L1 together with $CD33^{high}CD11b^{+}HLA\text{-}DR^{-/low}$. The MDSCs PD-L1+ population was calculated over the total number of acquired leukocytes, identified in the DCV+ gate (R1), in the single cell gate (R2) and in the 7-AAD- gate (R3) (data not shown). Changes in PD-L1 expression after PMA treatment found a 191-fold increase after stimulation (Panel B), compared with non-stimulated cells (Panel A), suggesting that fold-change in PD-L1 appears to have an important regulatory factor that may relate to differences in underlying mechanisms within patients.
Merging all measurements together into a single FCS file using a process called concatenation, peripheral blood samples obtained before and after immunotherapy treatment were effectively compared. Dimensionality reduction was performed on the concatenated data set. After PMA stimulation, PD-L1+ cells were displayed based on the expression of 6 markers using t-SNE (t-distributed stochastic neighbor embedding) visualization. The axes (t-SNE1 and t-SNE2) show dimensionless values that were assigned to individual cells by the t-SNE algorithm, which places PD-L1+ MDSCs that have similar expression profiles close to one another. In Panel C, the intensity of the coloring is relative to the cell density of PD-L1+ MDSCs. In Panel D, the intensity of the coloring is relative to the expression of PD-L1+ MDSCs. MDSCs from pre- and post-immunotherapy have similar cell density and expression patterns that identify them as well conserved entities across biologic therapy.

Author contribution statement

L.G.R., and J.P. performed the experiments. L.G.R., A.A.H, M.D.W., J.A.B., J.J., R.R. and J.P performed data interpretation, and drafted the manuscript. R.R. and J.P conceived the study, participated in its design, conducted most experiments, and contributed the manuscript equally (*).

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Declaration of Competing Interest

M.D.W. and J.A.B. work for Thermo Fisher Scientific, which is in the business of selling flow cytometers and flow cytometry reagents.

Acknowledgements

We thank CERCA Programme/Generalitat de Catalunya and The Josep Carreras Foundation for institutional support. The authors are also very grateful for their advice and technical support to Sergio Ramón, Víctor Querol, Clara Streiff, Paola Paglia, and Lluís Sainz from Thermo Fisher for all his comments and discussions on earlier work in this research field. Jordi Petriz also acknowledges the financial support from The Obra Social La Caixa and Thermo Fisher Scientific.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2020.100969.

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Revised 13 November 2020