Case Report

Characterization of Myeloid-derived Suppressor Cells in a Patient With Lung Adenocarcinoma Undergoing Durvalumab Treatment: A Case Report

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Clinical Practice Points

- Considering the wave of enthusiasm triggered by the recent introduction of immunotherapy in daily clinical practice for the treatment of lung cancer, a great effort from the scientific community has been dedicated to the identification and validation of reliable biomarkers able to drive the activity of immunotherapeutic agents.
- With this aim, we prospectively evaluated myeloid-derived suppressor cell frequency and functional activity during immunotherapy for lung cancer. In this patient with stage III non–small-cell lung cancer, durvalumab in maintenance promoted a strong reduction in both myeloid-derived suppressor cell1 frequency and activity, as well as in the expression of immunosuppression-associated genes.
- Our results provide the first evidence that immune checkpoint blockade may induce a comprehensive systemic alteration in the myeloid compartment in patients with lung cancer, probably through a progressive reduction of tumor burden and relief of tumor-induced immunosuppression. With this analysis, we demonstrated that tracking the immune environment is potentially feasible in daily clinical practice in order to characterize the dynamics of systemic immunologic factors during immunotherapy and evaluate their prognostic/predictive potential and applicability as surrogate markers for clinical response.

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Introduction

A 58-year-old woman diagnosed with stage IIIC lung adenocarcinoma was treated with carboplatin/paclitaxel and concomitant radiotherapy. Following Response Evaluation Criteria In Solid Tumors (RECIST) partial response, the patient was started on maintenance therapy with the anti-programmed death-ligand 1 (PD-L1) checkpoint inhibitor durvalumab (10 mg/kg dose every 2 weeks). Myeloid-derived suppressor cell (MDSC) frequency and functional activity were evaluated using the 'Cancer Immunoguiding Program' proficiency panel[1] on frozen peripheral blood mononuclear cells isolated from blood collected pre-treatment and after 6 weeks of immunotherapy (before the third durvalumab administration). To confirm MDSC functional properties, the patient's CD14+ monocytes were co-cultured in vitro with anti-CD3/CD28-activated, allogenic cell-trace-labeled T cells from buffy coats.[2] Lastly, expression levels of 4 MDSC-associated targets were evaluated by real-time polymerase chain reaction (PCR). Before durvalumab, the MDSC1 (CD14+CD124+ cells) subset was consistently increased compared with healthy donors (HDs) (n = 4) (mean value, 7.06% vs. 0.64% ± 0.33%). No alteration was reported in other MDSC subsets. In vitro functional assay

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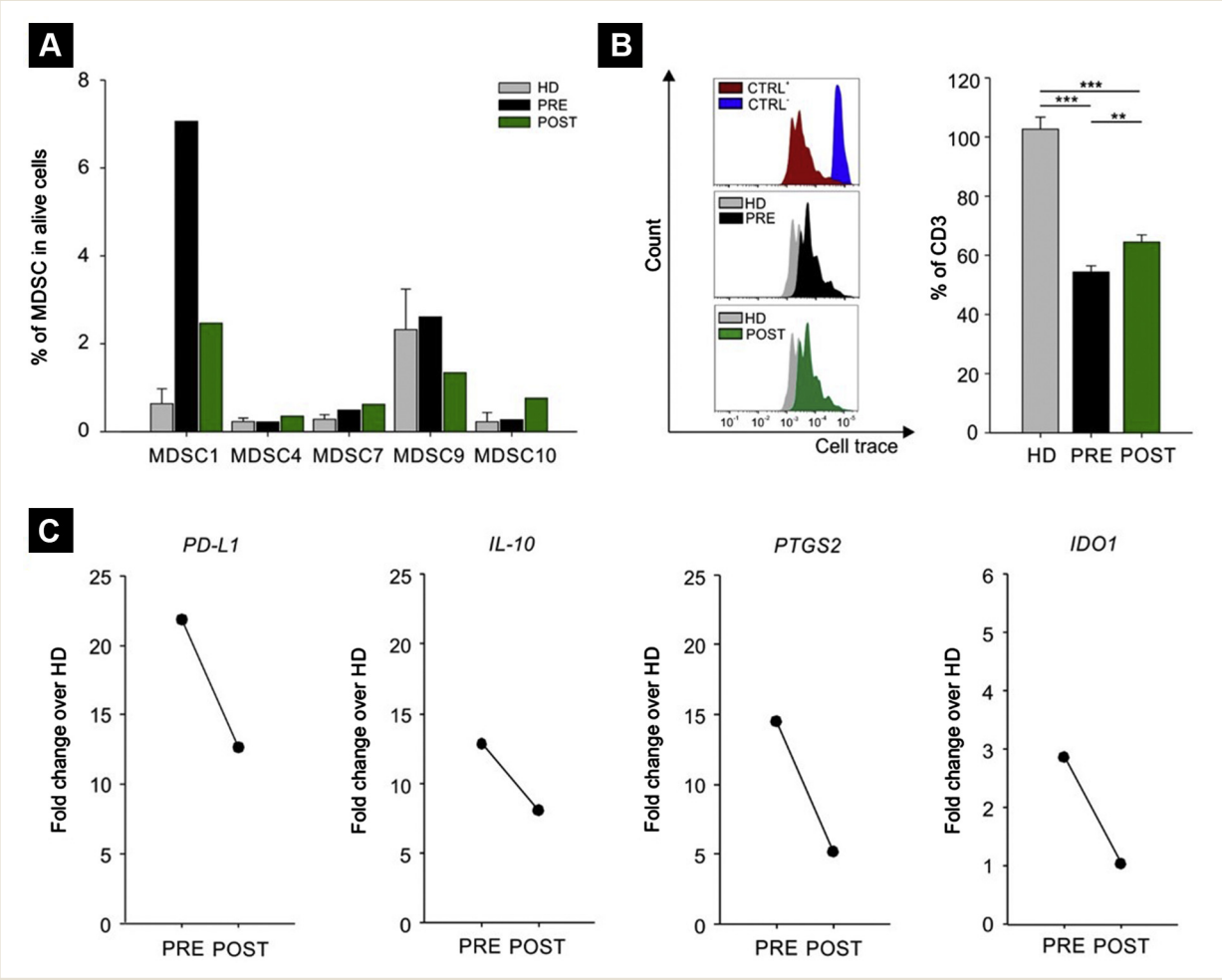
Figure 1 A, Flow Cytometry Analysis of MDSC Subsets (MDSC1: CD14+CD124+; MDSC4: CD14+HLA-DRlow/-; MDSC7: CD15-CD14+CD33highHLA-DRlow/-; MDSC9: CD14-CD15-CD33high; MDSC10: CD3-CD14-CD19-CD56-HLA-DRlow/-CD11b+) in Frozen Peripheral Blood Mononuclear Cells Isolated From Patient Before (Black) and During (Green) Immunotherapy Compared With HDs (Grey). B, Functional Assay Performed (at 1:3 Ratio of Allogeneic, Activated T Cells to CD14+ Cells) Using Frozen CD14+ Cells Isolated From Either Patient or HD. Data Are Reported as Percentage of CD3+ Proliferating Cells (Right Panel) and Graphed as Proliferation Peaks of Cell Trace+CD3+ Cells After the Co-culture Left Panel: CTRL+ (Red), Only Activated T Cells; CTRL− (Blue) Only Non-Activated T Cells; HD (Grey) Activated T Cells in Presence of HD-Derived CD14+ Cells; PRE (Black) and POST (Green) Activated T Cells in Presence of Patient's CD14+ Cells Before and After Immunotherapy, Respectively. Data Are Shown as Mean ± SD of Experimental Triplicates. Statistical Analysis Was Performed Using the Analysis of Variance Test: $^{**}P < .01$, $^{***}P < .001$. C, Expression Levels of MDSC-Associated Genes (PD-L1, IL-10, PTGS2, IDO1) Were Evaluated by Real-time Polymerase Chain Reaction (RT-PCR). RT-PCR Was Run Using 2× SYBR Greenmaster Mix (ABI). All Samples Were Normalized Using Glyceraldehyde 3-Phosphate Dehydrogenase Endogenous Control Primers. Post-Quantitative RT-PCR Analysis to Quantify Relative Gene Expression Was Performed by the Comparative Ct Method (2−ΔΔCt) Over HD Values



Abbreviations: CD = cluster of differentiation; CTRL = control; HD = healthy donors; HLA = human leukocyte antigen; IDO1 = indoleamine 2,3-dioxygenase 1; IL-10 = interleukin 10; MDSC = myeloid-derived suppressor cells; PD-L1 = programmed death-ligand 1; POST = after immunotherapy; PRE = before immunotherapy; PTGS2 = prostaglandin-endoperoxide synthase 2.

demonstrated that patient's CD14+ cells exhibited a strong immunosuppressive activity compared with CD14+ cells isolated from HD. In fact, we observed a strong contraction in T cell proliferation when activated lymphocytes were co-cultured in the presence of patient's CD14+ cells (mean value of proliferation, 54.3 ± 2.09 vs. 102.65 ± 4.03; $P < .001$). Furthermore, all 4 MDSC-associated genes were overexpressed in pretreatment samples as compared with HD: *PD-L1* (22-fold), *PTGS2* (prostaglandin-endoperoxide synthase 2) (15-fold), *IL-10* (interleukin-10) (13-fold), and *IDO-1* (indoleamine 2,3-dioxygenase 1) (3-fold). After 2 durvalumab administrations, the patient's MDSC1 subset dropped approximately 3 folds (from 7.06% to 2.47%), and the functional assay showed an impairment in their immunosuppressive activity. Indeed, even if the patient's CD14+ cells showed inhibitory properties compared with HD (mean value of proliferation, 64.4 ± 2.4 vs. 102.65 ± 4.03; $P < .001$), their immunosuppressive

impact was significantly compromised compared with pretreatment (pretreatment vs. posttreatment: mean value of proliferation, 54.3 ± 2.09 vs. 64.4 ± 2.4; $P < .06$). Finally, expression of the evaluated genes was decreased: 12-fold as compared with baseline for *PD-L1*, 5-fold for *PTGS2*, 8-fold for *IL-10*, and 1-fold for *IDO1* (Figure 1). After 6 months of durvalumab, the patient maintains a clinical and radiologic disease remission.

Discussion

MDSCs are a mixed cell subset of myeloid cells characterized by morphologic, phenotypic, and functional heterogeneity.[3] Their main feature is the ability to inhibit immune response. MDSC accumulation in patients' peripheral blood is induced by several inflammation- and tumor-derived factors,[4] and their frequency has been related to poor survival in several tumors, including non−small-cell lung cancer,[5] as well as to poor response to ipilimumab therapy in melanoma.[6] Despite the morphologic and clinical remission induced by radio-chemotherapy, immunologic profiling demonstrated profound systemic immunosuppression in the studied patient, as testified by the presence of a relevant number of circulating, functional MDSCs. Durvalumab promoted a strong reduction in both MDSC1 frequency and activity, as well as in the expression of immunosuppression-associated genes. These results raise the intriguing possibility that immune checkpoint blockade may induce a critical systemic alteration in the myeloid compartment,[6] through further reduction of the tumor burden and relief of tumor-induced immunosuppression. Moreover, we demonstrated the feasibility of tracking the immune environment in daily clinical practice. In the future, we will evaluate MDSC fluctuation in the blood of immunotherapy-treated patients in a more restricted time frame to clarify if the MDSC contraction may anticipate the clinical response. With this aim, we will apply also new MDSC-associated markers, such as the expression of cellular FLICE (FADD-like IL-1β-converting enzyme)-inhibitory protein, which we recently validated as a potential useful tool to refine the immunologic landscape of patients with cancer.[7] In addition, to clarify the potential influence of chemotherapy and/or radiotherapy on circulating immune cells and to validate these preliminary findings, we are currently expanding the analysis to different stages of lung cancer and including other malignancies treatable with immunotherapy, such as melanoma and renal cancer. These prospective, longitudinal studies are warranted to characterize the dynamics of systemic immunologic factors, including MDSC profiling during immunotherapy, and evaluate their prognostic/predictive potential and possible application as surrogate markers for clinical response.

Conclusion

To our knowledge, this represents the first evidence that immune checkpoint blockade may induce a critical systemic alteration in the myeloid compartment in patients with lung cancer, potentially trackable in daily clinical practice.

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Disclosure

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