Overexpression of PD-L1 Correlates with JAK2-V617F Mutational Burden and Is Associated with Chromosome 9p Uniparental Disomy in MPN

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62nd Annual ASH Meeting
December 6th, 2020
Disclosures

The presenting author and all other co-authors not listed below have nothing to disclose.

Heinz Gisslinger, MD: Honoraria and Research Funding – AOP Orphan Pharmaceuticals AG & Novartis; Honoraria – PharmaEssentia, MyeloPro Diagnostics and Research, Janssen-Cilag, Roche & Celgene

Robert Kralovics, PhD: Honoraria – AOP Orphan Pharmaceuticals AG, PharmaEssentia, Qiagen & Novartis; Current equity holder in a private company - MyeloPro Diagnostics and Research

Peter Valent, MD: Research Funding – Allcyte Gmbh; Honoraria – Pfizer; Honoraria and Research Funding – Cellgene

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Background and Objectives

Myeloproliferative neoplasms (MPN) are characterized by clonal hematopoiesis, hyperproliferation of myeloid cells, hyperinflammation and immune deregulation. The three classical \textit{BCR-ABL1}-negative MPN are essential thrombocythemia, polycythemia vera and primary myelofibrosis. The disease is driven by \textit{JAK2}, \textit{CALR} or \textit{MPL} somatic mutations in most patients. Drug resistance is a major problem in MPN. Recent data suggest that MPN cells display certain immune checkpoint molecules that may contribute to resistance, including PD-L1. Antibodies targeting the PD1/PD-L1 axis are highly promising anti-cancer drugs. Their potential use in MPN is being explored but it is unclear which MPN subtypes are most suitable for testing in clinical trials.

The aim of our project was to assess PD-L1 expression in disease-initiating neoplastic stem cells and differentiated cells of MPN patients and to develop therapeutic approaches capable of blocking PD-L1 expression in MPN stem cells.
RNA-Sequencing Reveals Overexpression of PD-L1 in Neoplastic Cells of Polycythemia Vera Patients

*PD-L1* expression was assessed by RNA-sequencing of granulocytes of 106 MPN patients and 15 healthy donors (A). We observed a ~5-fold higher expression of *PD-L1* mRNA in patients with PV compared to other MPN (P<.01) or healthy controls (P<.01) (B). *JAK2-V617F* positive ET patients had higher expression of *PD-L1* compared to *CALR*-mutated ET (p<.005) and the same was observed in PMF (p<.01) (C). Other mutations (*TET2, DNMT3A*) detected by NGS did not affect *PD-L1* expression.

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Overexpression of PD-L1 Is Associated with 9p Uniparental Disomy in MPN

PD-L1 and JAK2 are both affected with 9p UPD in 195 MPN patients who carry the aberration in the cohort of 400 MPN patients analyzed by Affymetrix Human Genome-Wide 6.0S SNP arrays. As PD-L1 is more centromeric it could represent the second target of 9pUPD which can precede the acquisition of JAK2-V617F in MPN (A).

Granulocytes in JAK2-V617F positive patients with 9pUPD expressed significantly higher levels of PD-L1 compared to patients without 9pUPD (B), and the same was observed when the patients were stratified by diagnosis (C).
The JAK2-V617F mutational burden significantly correlated with PD-L1 expression in MPN patients ($R=.52$, $P<.0001$; A). This correlation was lost when cases with 9pUPD were excluded from the analysis ($R=.03$, $P=.9$; B), indicating that the presence of the chromosomal aberration is relevant for PD-L1 upregulation.
PD-L1 is Overexpressed on the Cell Surface of Putative Neoplastic Stem Cells in MPN

PD-L1 surface expression on CD34+CD45dimCD38- cells isolated from fresh bone marrow (BM) samples of another 51 MPN patients and 7 healthy controls was assessed by flow cytometry (A, C). PD-L1 levels on the SC surface were elevated in both JAK2- and CALR-mutated MPN patients compared to healthy donors (B). CD4+ and CD8+ T-cells from BM samples of 17 MPN patients expressed the PD-L1 receptor PD-1 (D). PD-L2 was neither expressed in MPN granulocytes nor on MPN SC (E).

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Ruxolitinib and dBET6 Block IFN-γ-Induced PD-L1 Expression in CD34⁺CD45\textsuperscript{dim}CD38⁻ MPN Stem Cells

We cultured \textit{ex vivo} primary MPN cells from 7 JAK2-V617F positive patients and showed that PD-L1 expression on MPN stem cells spontaneously decreases in culture, that interferon-gamma (IFN-γ) can promote expression of PD-L1 on these cells, and that ruxolitinib and the BRD4-degrader dBET6 block IFN-γ-induced PD-L1 expression in CD34⁺CD45\textsuperscript{dim}CD38⁻ MPN stem cells (P<.05).

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Conclusions

• **PD-L1** mRNA is overexpressed in granulocytes of MPN patients and **PD-L1** overexpression in granulocytes correlates with the **JAK2-V617F** mutational burden.

• In patients with **JAK2-V617F** positive MPN, 9pUPD leads to further **PD-L1** upregulation.

• **PD-L1** is overexpressed on the surface of putative neoplastic stem cells in MPN patients.

• T cells of MPN patients express **PD-L1** receptor PD1.

• Ruxolitinib and dBET6 treatment lead to downregulation of **PD-L1** expression on MPN stem cells suggesting a role for the JAK2 and BRD4-MYC pathway.
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