

## THE IL8-CXCR1/CXCR2 AXIS PLAYS A ROLE IN THE MOBILIZATION PROCESS OF POLYMORPHONUCLEAR MYELOID-DERIVED SUPPRESSOR CELLS IN PATIENTS WITH PRIMARY MYELOFIBROSIS

R. Campanelli<sup>1</sup>, S. Mantovani<sup>2</sup>, A. La Gaipa<sup>2</sup>, C. Ramirez<sup>3</sup>, C. Abbà<sup>4</sup>, A. Carolei<sup>1</sup>, P. Catarsi<sup>1</sup>, C. Del Fante<sup>5</sup>, G. Barosi<sup>1</sup>, M. Massa<sup>4</sup> And V. Rosti<sup>1</sup>

<sup>1</sup>Center for the Study of Myelofibrosis, Istituto di Ricovero e Cura a Carattere Scientifico Policlinico San Matteo Foundation, Pavia; <sup>2</sup>Department of Translational and Clinical Research, Laboratory of Clinical Immunology, Istituto di Ricovero e Cura a Carattere Scientifico Policlinico San Matteo Foundation, Pavia; <sup>3</sup>Laboratory of RNA and Disease Data Science, Department of Cellular, Computational and Integrative Biology, University of Trento, Trento; <sup>4</sup>General Medicine 2-Center for Systemic Amyloidosis and High-Complexity Disease, Istituto di Ricovero e Cura a Carattere Scientifico Policlinico San Matteo Foundation, Pavia; <sup>5</sup>Division of Immunohaematology and Transfusion Service, Istituto di Ricovero e Cura a Carattere Scientifico Policlinico San Matteo Foundation, Pavia.

**Introduction:** PMF is a myeloproliferative neoplasm characterized by a chronic inflammatory state that contributes to the disease pathogenesis. MDSCs are immature myeloid cells that expand in patients with cancer, sepsis or chronic inflammation favoring tumor onset and progression mainly through the decrease in immune surveillance and the promotion of neoangiogenesis. We previously found that the PMN-MDSC subset is increased both in the circulation and in the spleen of PMF patients and strongly correlates with disease progression (Campanelli R, 2024). Although the precise mechanism underlying the egress of MDSCs from the bone marrow (BM) is unknown, some papers suggest that migration of neutrophils and MDSCs is due to CXC chemokines and inflammatory cytokines. Aim of the study is to investigate the role of IL8 and its receptors (CXCR1, CXCR2) in the mobilization of PMN-MDSCs in PMF.

**Methods:** PMN-MDSCs were identified, sorted by FACS and sequenced by Illumina using the Stranded Total RNA Prep, Ligation with Ribo-Zero Plus Kit. We analysed PMF patients (n=8) and G-CSF-mobilized healthy subjects (G-HDs; n=7). We also measured the membrane expression of CXCR1/2 on PMN-MDSC as percentage and mean fluorescence intensity (MFI) (see Figure).

**Results:** In PMN-MDSCs, the gene expression analysis, expressed as FKPM, showed that IL8 was highly expressed ( $p<0.01$ ) in G-HDs (median 1458, range 405-3923) with respect to PMF (median 207, range 65-642). Moreover, the

gene expression of CXCR1 and CXCR2 was higher ( $p=0.01$  for both) in G-HDs (median; range: 211; 108-403 and 172; 86-469, respectively) than in PMF (median; range: 37; 22-75 and 22; 6-39, respectively).

The receptor analysis showed that CXCR1-expressing PMN-MDSCs were significantly higher both in PMF ( $p<0.001$ ) and in G-HDs ( $p<0.02$ ) with respect to HDs (Figure panel A). Despite being elevated, CXCR2-expressing PMN-MDSCs were not different in PMF, G-HDs and HDs (panel C). The MFI of CXCR1 of PMN-MDSCs was higher but not statistically different in G-HDs and PMF than in HDs (panel B), while CXCR2 MFI was comparable in PMF, G-HDs and HDs (panel D). Although an aberrant expression of IL8 has been reported in various pathologies including myeloproliferative neoplasms (Ramachandra N 2023), we will assess this chemokine in the subjects involved in the study.

**Conclusions:** The overexpression of CXCR1 and its relationship with the number of circulating PMN-MDSCs in PMF indicates an involvement of IL8-CXCR1/2 in the mobilization process that leads the release of these cells from the BM, as it happens in G-HDs. The overexpression of IL8-CXCR1/2 was previously associated with poor prognosis in hematological and solid tumors and CXCR1/2 is the target of ongoing clinical trials in patients with MDS, melanoma, prostate, breast and lung cancers. In this regard, our results suggest that IL8-CXCR1/2 axis can be a potential therapeutic target also in PMF to reduce PMN-MDSCs, known to play a role in the disease worsening.

MYELOPROLIFERATIVE DISORDERS

**Figure.** Membrane expression of CXCR1 (panels A, B) and CXCR2 (panels C, D) on PMN-MDSCs, obtained from PBMCs of PMF patients (n=17), G-CSF-mobilized healthy subjects (G-HDs; n=6) and healthy donors (HDs; n=7). PMN-MDSCs were identified as CD15<sup>+</sup>CD11b<sup>+</sup>Lox1<sup>+</sup> and the expression of the receptors was evaluated as percentage (panels A, C) and mean fluorescence intensity (MFI) (panels B, D).

