# 1212 Interferon-γ-Dependent Inflammatory Signature in Acute Myeloid Leukemia Cells Is Able to Shape Stromal and Immune Bone Marrow Microenvironment

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***Marilena Ciciarello, PhD***[1*], *Giulia Corradi, PhD*[2*], *Sabina Sangaletti, PhD*[3*], *Barbara Bassani*[3*], *Giorgia Simonetti, PhD*[4*], *Jayakumar Vadakekolathu, PhD*[5*], *Giovanni Marconi*[1], *Giovanni Martinelli, MD*[6*], *Mario Paolo Colombo*[3*], *Sergio Rutella, MD, PhD, FRCPath*[7,8], *Michele Cavo*[1*] *and Antonio Curti, MD, PhD*[9]

[1]*Azienda Ospedaliero-Universitaria S. Orsola-Malpighi, Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Institute of Hematology L. e A. Seràgnoli, Bologna, Italy*
[2]*Department of Experimental, Diagnostic and Specialty Medicine, Institute of Hematology "L. and A. Seràgnoli", University of Bologna, Bologna, Italy*
[3]*Fondazione IRCCS Istituto Tumori, Milan, Italy*
[4]*Biosciences Laboratory, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola (FC), Italy*
[5]*John van Geest Cancer Centre School of Science and Technology, Nottingham Trent University, Nottingham, United Kingdom*
[6]*Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola (FC), Italy*
[7]*Centre for Health, Ageing and Understanding Disease (CHAUD), Nottingham Trent University, Nottingham, United Kingdom*
[8]*John van Geest Cancer Research Centre, Nottingham Trent University, Nottingham, United Kingdom*
[9]*Department of Hematology and Oncology Institute of Hematology L. e A. Seràgnoli, Azienda Ospedaliero-Universitaria S. Orsola-Malpighi, Bologna, Italy*

## Background

Acute myeloid leukemia (AML) has been considered for a long time exclusively driven by critical mutations in hematopoietic stem cells (HSCs). Recently, the contribution of bone marrow (BM) microenvironment has gained increasing attention, challenging the evidence that AML derives exclusively from leukemic cell-intrinsic defects. Mesenchymal stromal cells (MSCs) are a key component of the BM microenvironment by regulating HSC fate and having a unique immune-modulatory capacity mostly mediated by interferon (IFN)-γ-induced indoleamine 2,3-dioxygenase (IDO)-1 enzyme activity. New studies demonstrated that the alterations of MSCs are able (e.g. by promoting an inflammatory/genotoxic microenvironment) to induce hematological diseases in mice models and humans. Moreover, AML cells seem to exploit MSC-dependent pro-survival signals to their advantage. All these concepts converge to indicate a fundamental bi-directional interaction among malignant cells and BM microenvironment contributing to AML onset and progression. The mechanisms underlying this crosstalk have just been started to get unraveled. Among signals potentially driving the remodeling of the BM microenvironment, inflammation, a hallmark of cancer, seems to play a role. We hypothesize that 'inflammatory features' of leukemic cells can shape MSCs by inducing functional changes able to create a permissive/self-reinforcing niche favorable to escape therapy and immune response.

## Methods

We isolated acute myeloid leukemia (AML) cells and generated AML-MSCs from the BM of AML patients. Gene expression profile (GEP) (AML, N=61; healthy donors, HDs N=7) and NanoString analysis (AML, N=24) on BM-derived cells were also done. Next, we set up AML-MSC/AML cell co-culture experiments and we investigated gene expression in AML-MSCs and AML cells before and after co-cultures. We also set up a murine model in which the IFN-γ expressing C1498 AML cells was knock down (KD) for the IFN-γ gene by RNA interference. BM infiltrate was analyzed in mice and AML patients.

Results

In a GEP-screening, we found that almost 40% of AML samples showed an IFN-γ expression higher than the median level of IFN-γ expression in HDs. NanoString data and pathway analysis indicated that IFN-γ high AML cells (above the median level) presented an inflammatory/immune modulating signature clearly distinct from IFN-γ low AML cells (below the median level). Moreover, IFN-γ expression in AML samples correlated with the up-regulation of IFN-γ-stimulated genes (ISGs) (e.g. IDO-1, Programmed death-ligand (PDL)-1 and Nitric Oxide synthase (NOS)-2), which are known to regulate immunity and tolerance. Thus, we aimed to gain insights into IFN-γ-dependent modifications in the leukemic milieu. In AML-MSC/AML cell co-culture experiments, we detected that AML cells produced IFN-γ. To gain insight in AML cell-dependent MSC modifications, we analyzed ISG expression in MSCs, after co-cultures with IFN-γ high or IFN-γ low AML cells. We found that IFN-γ high, but not IFN-γ low AML cells, were able to induce IDO-1, PDL-1 and NOS-2 in AML-MSCs. Moreover, ISG upregulation was abrogated by an IFN-γ neutralizing antibody. We also found that AML-MSCs, after co-culture with IFN-γ high AML cells, were able to induce regulatory T cells (Tregs) in an IDO1-dependent manner. *In vivo* experiments showed a higher percentage of engraftment in immunocompetent mice injected with parental IFN-γ expressing cells compared to mouse injected with the KD counterpart. The take of parental C1498 cells was associated to an increased frequency of Tregs in the BM. Furthermore, the BM microenvironment of mice injected with IFN-γ KD-C1498 cells showed a significant reduction of PD-L1 expressing cells. Consistently, BM infiltrate analysis in AML patients showed that the percentage of Tregs was correlated with the percentage of AML IFN-γ-positive cells in the BM.

Conclusion

Our data suggest that interferon-γ-dependent inflammatory signals produced by AML cells are able to modify MSC functions, thus favoring an immune modulating and leukemia-supporting milieu. Overall, our results unravel MSC-dependent mechanisms that might promote leukemia resistance to therapy, therefore informing the delivery of novel therapies targeting the AML microenvironment such as IDO inhibitors and immune checkpoint blockade.