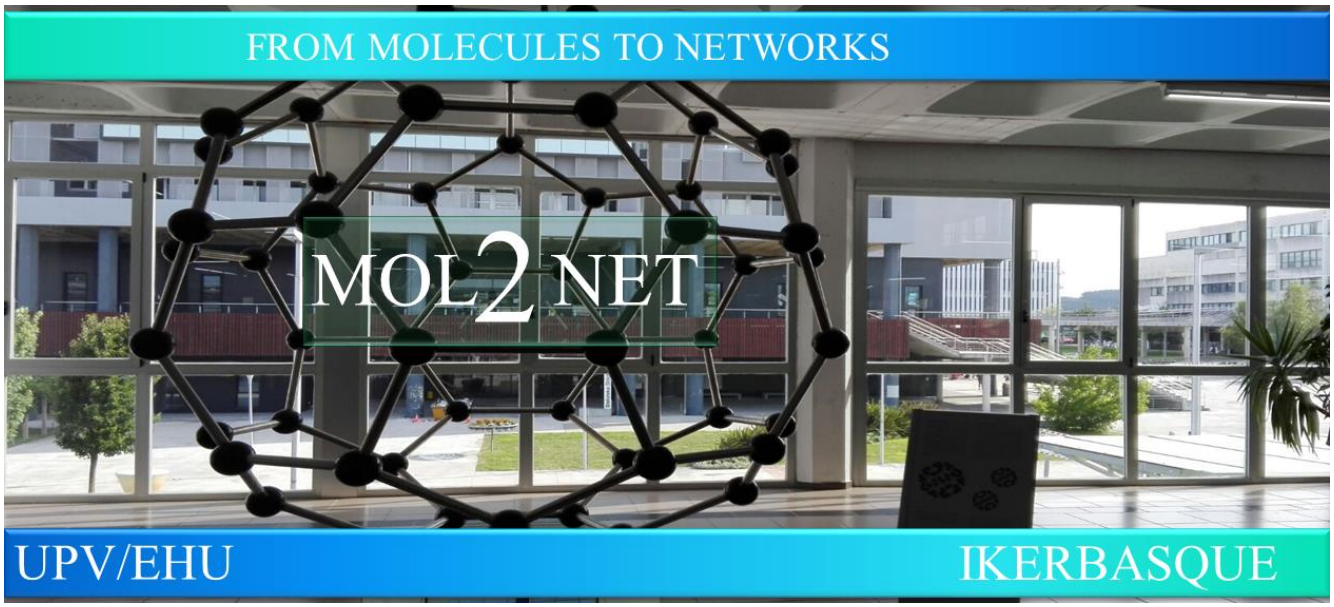
MDPI sciforum

**MOL2NET'21, Conference on Molecular, Biomedical & Computational Sciences and Engineering, 7th ed.**

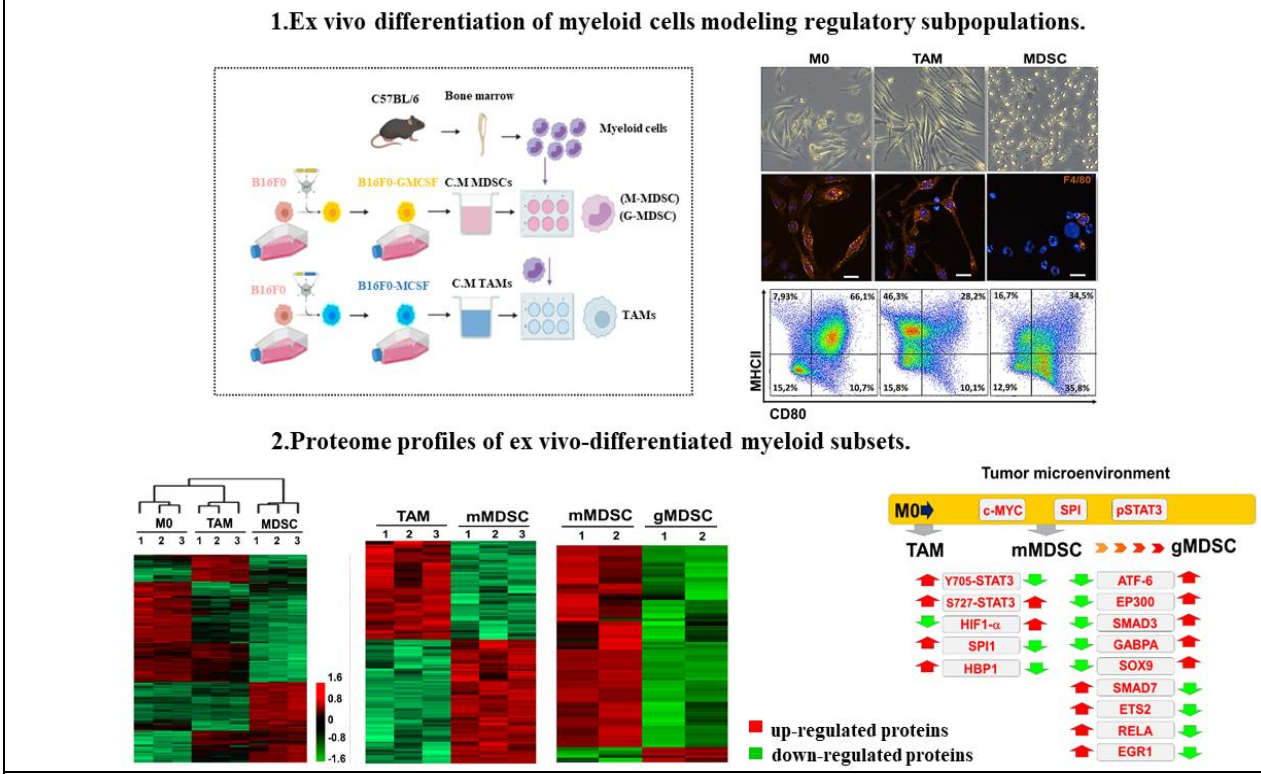



# Proteomic analysis of murine tumor associated myeloid populations for myeloid cell reprogramming in cancer

*Ester* Blanco[1], *Luisa Chocarro*[1], Hugo *Arasanz*[1,2], *Ana Bocanegra*[1], *Leticia Fernández-Rubio*[1], *Miriam Echaide*[1], *Maider Garnica*[1], *Pablo Ramos, Cristian Smerdou* [3], *David Escors*[1] *Grazyna Kochan*[1],

[1]Oncoimmunology Research Unit, Navarrabiomed-Fundación Miguel Servet, Universidad Pública de Navarra (UPNA), Complejo Hospitalario de Navarra (CHN), Instituto de Investigación Sanitaria de Navarra (IdiSNA), Pamplona, Spain.
[2]Medical Oncology Unit, Complejo Hospitalario de Navarra (CHN), Instituto de Investigación Sanitaria de Navarra (IdiSNA), Pamplona, Spain.
[3] *Division of Gene Therapy and Regulation of Gene Expression, Cima Universidad de Navarra and Instituto de Investigación Sanitaria de Navarra (IdISNA), Pamplona, Spain*

## Graphical Abstract




**Abstract.**

Tumor microenvironment (TME) remodeling is one of the major research subjects in oncology. Several strategies can be implemented to modulate the tumor microenvironment, particularly in reprogramming myeloid cells to stimulate their anti-cancer activities. Indeed, myeloid cells constitute the major component of TME. Hence, it is important to identify the molecular signatures associated to cancer-promoting myeloid cells. Here, we defined the phenotype and proteome of tumor associated myeloid cells. Moreover, we identified the relationships between myeloid-derived suppressor cells (MDSCs) and tumor associated macrophages (TAM). The proteomic atlas of tumor-associated cells revealed important routes to reprogram cancer-associated myeloid cells.

## Introduction

Tumor associated myeloid cells are major promoters of progression and metastasis. Monocytic and granulocytic myeloid-derived suppressor cells (M-MDSC and G-MDSC) together with tumor associated macrophages (TAM) are among the main contributors to tumor-induced immunosuppression. The influence of the tumor microenvironment on their differentiation is well-accepted but the specific molecular changes leading towards MDSC subsets and TAM are not well-characterized.

## Materials and Methods

We used an *ex vivo* differentiation system for MDSCs and TAM by from C57BL/6J mouse bone marrow cells in cancer-polarized conditioning medium (1). Three global experiments of quantitative mass spectrometry (shotgun proteomics) were performed. Construction of functional interactomes maps from up- or down-regulated proteins was conducted with the Ingenuity Pathway Analysis (IPA) Tool from Quiagen. Targeted proteins were evaluated using western blot. We evaluated the effect at differentiation,

maturation and immunosuppressive level in MDSCs and TAMs of several compounds. Markers were evaluated by cytometry and western blot.

## Results and Discussion

We confirmed morphological and phenotypic differences in *ex vivo* differentiated myeloid populations. High-throughput proteomics uncovered protein expression patterns characteristic of populations modelling tumor-infiltrating subsets, as a result of cancer-derived factors.

Therefore, we evaluated several compounds for reprogramming tumor-associated cells. A resemblance to activated myeloid cells and a greater rise of macrophages and DC were observed. Among the several changes obtained, here we highlight antigen presentation markers, they showed increases only with compound 1. In addition, a greater rise of macrophages and DC was observed and the secretion of inflammatory IL12 cytokine was induced (Figure 1b and 1c). Moreover, the immunosuppressive functions of MDSCs decreased which led to enhanced CD4 cells proliferation and increased CD4 ability to release more IFN-gamma and IL2 (Figure 1d).

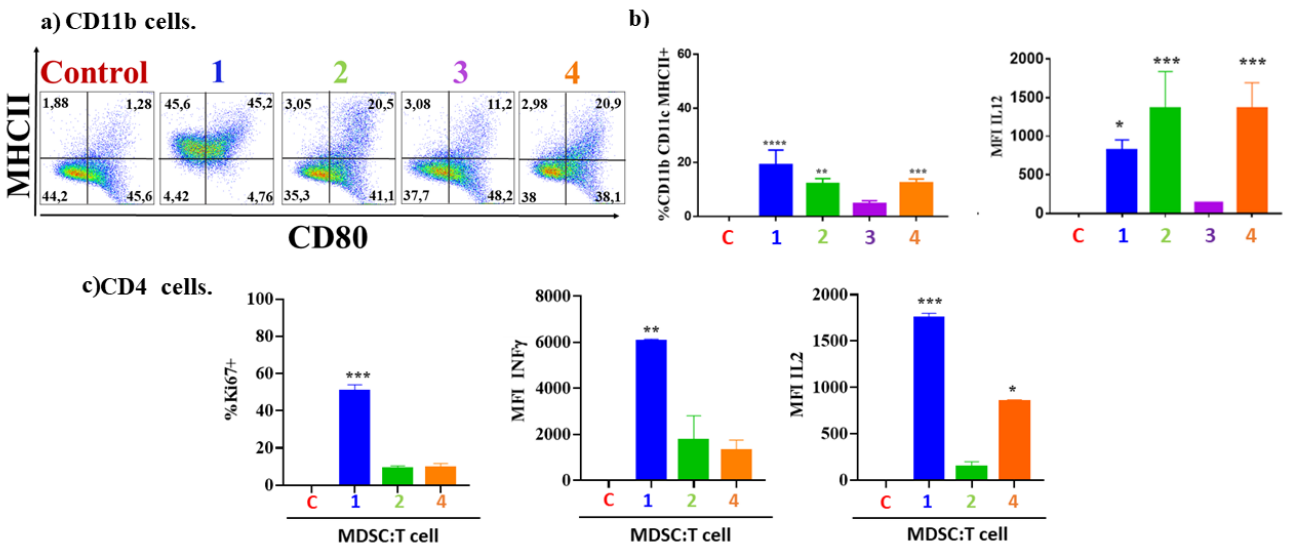



**Figure 1: Targeting the main routes of MDSCs ex-vivo differentiated cells.** a) Flow cytometry density plots of MHCII and CD80 expression in differentiated MDSCs. The gates were established according to unstained control cells. b) Percentage of MDSCs *ex vivo* differentiated that express CD11b Cd11c MHCII and IL12 of MDSCs normalized to the control. (N=3). c) Percentage of CD4 cell proliferation, INFγ and IL2 (MFI) in co-culture of MDSCs. Colors of the bars in figure b, c and d correspond to color of the compounds 1, 2,3 and 4 (1 – blue; 2 – green; 3 – purple; 4 – orange).

## Conclusions

In the present study, we identified differences in proteomic signatures between MDSCs and TAMs related to lineage, and cancer-driven polarization. Moreover, these result permit us develop strategies to reprogram myeloid cells cancer associated.

**References**

1) Liechtenstein T, Perez-Janices N, Gato M, et al. A highly efficient tumor-infiltrating MDSC differentiation system for discovery of anti-neoplastic targets, which circumvents the need for tumor establishment in mice. Oncotarget. 2014;5(17):7843-7857. doi:10.18632/oncotarget.2279

2) Blanco E, Ibañez-Vea M, Hernandez C, et al. A Proteomic Atlas of Lineage and Cancer-Polarized Expression Modules in Myeloid Cells Modeling Immunosuppressive Tumor-Infiltrating Subsets. J Pers Med. 2021;11(6):542. Published 2021 Jun 11. doi:10.3390/jpm11060542