Targeting the Immunomodulatory Capacity of MDS MSCs by Tasquinimod

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Motivation

Myelodysplastic syndromes (MDS) belong to the most common hematological neoplasms in the elderly population, characterized by **ineffective hematopoiesis**, **peripheral cytopenia**, and **the risk of transformation into acute myeloid leukemia**. A dysregulated innate immune response and **proinflammatory bone marrow (BM) microenvironment** play a crucial role in the MDS pathogenesis by providing chronic inflammation which makes those pathways the perfect candidate for future therapeutics. Specifically, it has been shown that the **alarmin S100A9**, an important ligand for driving inflammation and promoting tumor progression, is **elevated** in MDS patients. Previous experiments performed in Stem Cell Lab 2 provided evidence that mesenchymal stromal cells (MSCs), an important component of the BM niche with immunomodulatory capacity, can be targeted by the novel oral small molecular drug **Tasquinimod (TASQ, Active Biotech)** which has demonstrated **S100A9 inhibitory activity**. However, almost nothing is known about the **potential effects of TASQ in the context of immunomodulation**.

Aim

The aim of this project is to **understand the effects of TASQ on the immunomodulatory capacity of Mesenchymal Stromal Cells (MSCs) obtained from MDS patients in response to T cell-mediated inflammation** through the evaluation of critical **adhesion (ICAM1, VCAM1)**, **immune checkpoint (PDL1, PDL2)**, **anti-inflammatory cytokine (COX2, IDO1)**, **chemokines (CCL2, IL8)** known to be upregulated and **extracellular matrix-related (COL4A2, COL1A1)** known to be downregulated under inflammation.

Methods

- mRNA expression analysis with quantitative real-time PCR
- Protein expression analysis with Flow cytometry
- Analysis of TASQ effect on co-cultured PBMCs via MTT, ELISA, and FACS

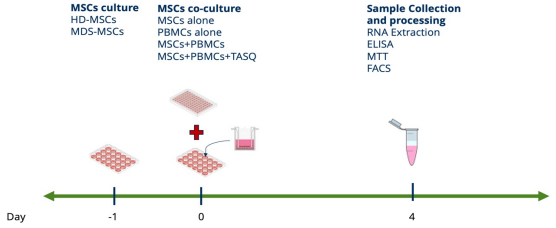

Figure 1 – Methodology of the research project

Result

TASQ treatment decreases the expression of important immunomodulatory genes in MSCs at the mRNA level.

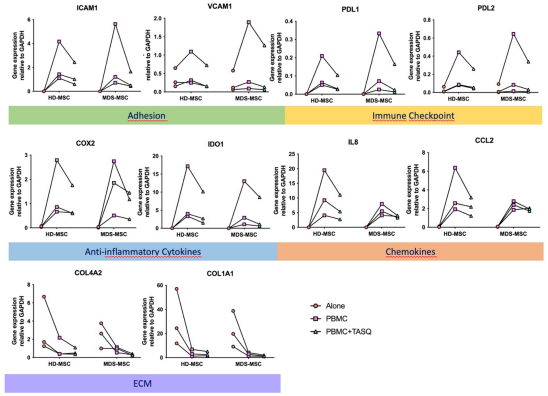

Figure 2 - MSCs obtained from 3 healthy donors (HD-MSC) and 3 MDS patients (MDS-MSC) were co-cultured with stimulated PBMCs with or without TASQ treatment at a PBMS: MSC ratio of 10:1. PBMCs and MSCs were also cultured alone. After 4 days of incubation, MSC cells were collected, RNA was isolated and transcribed into cDNA. Later, by using primers given in Supplementary Table 1, gene expression levels were measured via qPCR. Each line represents the data from a single donor and their gene expressions are shown relative to GAPDH. (N=2)

Conclusion

In conclusion, the preliminary findings are promising, although the differences between donors and insufficient number of repetitions of some experiments make it difficult to make a final judgment. In addition, the lack of significant changes made us think about the necessity of adjusting the incubation time and drug dose. But overall, these results are a clue that TASQ can alter the immunomodulation character of MSCs due to observed changes in mRNA and protein levels. However, changes that is noted in gene expression weren't sufficient to induce strong functional changes in MSCs the overall immunomodulatory potential of MSCs in this experimental setup upon TASQ treatment. Nevertheless, performing experiments with more donor groups, at different doses and time points will contribute to our understanding of the effect of TASQ on the immunomodulation of MSCs.

PBMC viability and pro-inflammatory cytokine IFN secretion is not affected by TASQ treatment, while IL-2 release is.

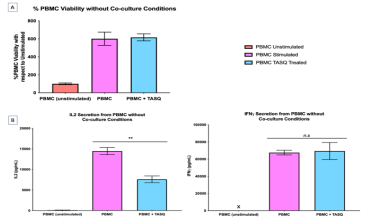

Figure 3 - (A) After 4 days of incubation, 100 uL PBMCs with or without TASQ treatment from monoculture were collected for cell proliferation assay. Results were measured at 570nm with a reference wavelength of 690nm. %Viability is given in terms of (sample OD/average of control OD)x100. (B) By also collecting 180 uL PBMCs at the same time, cells were removed by centrifugation and an ELISA assay for IL-2 and IFNγ was performed as mentioned in the method. Measurements were taken in Optical Density (OD). Data were analyzed by using GraphPad Prism. (A) mean of biological replicates± SEM, ns p>0.05, Tukey's multiple comparisons test, one-way Anova, n=2 (B) mean of biological replicates± SEM, ns p > 0.05, **p < 0.01, Tukey's multiple comparisons test, one-way Anova. n=1 for IL-2 and n=2 for IFNγ.)

PBMC proliferation in MSC co-cultures is unchanged when treated with TASQ, while proinflammatory cytokine release is altered.

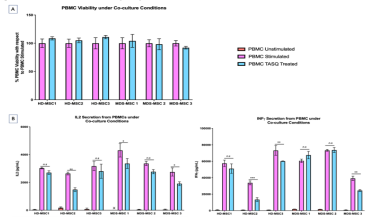

Figure 5 -At day 4, PBMC samples with or without TASQ treatment from co-cultured PBMCs with 3 healthy donors (HD-MSC1-3) and 3 MDS patients (MDS-MSC1-3) were collected for both proliferation assay (100uL) and cytokine analysis (180uLL). (A) For cell proliferation assay, results were measured at 570nm with a reference wavelength of 690nm. %Viability is given in terms of (sample OD/average of control OD)x100. (B) Cells were removed by centrifugation and ELISA assay for IL-2 and IFNγ was performed as mentioned in method. Measurements were taken in Optical Density (OD). Data were analyzed by using GraphPad Prism. (A) mean of biological replicates± SEM, ns p>0.05, Šídák's multiple comparisons test, two-way Anova, n=2 (B) mean of biological replicates± SEM, ns p > 0.05, *p < 0.05, **p < 0.01, ***p < 0.001, Tukey's multiple comparisons test,one-way Anova. n=1 for IL-2 and n=2 for IFNγ.)

Surface markers (ICAM1, VCAM1, PDL-1, and PDL-2) were minimally or not affected at the protein level by TASQ treatment

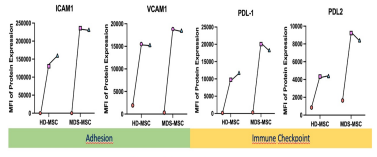

Figure 4 - 4 days co-cultured MSCs from healthy (N=1) and MDS donors (N=1) with PBMCs and with or without TASQ treatment were detached from the plate and were resuspended first with specific antibodies and then with DAPI by following the washing steps. Gating was done according to size (FSC vs SSC), single cell disassociation (FSC-A vs FSC-H), and live cell discrimination (DAPI). Later MFI (Median Fluorescence Intensity) was read out according to antibody-specific fluorochrome channels. (ICAM1- APC/Fire 750, VCAM1- PE-Cy7, PDL1-APC, PDL2-PE, n=1)

CD4+ and CD8+ T Cells are not rescued from senescence upon TASQ treatment

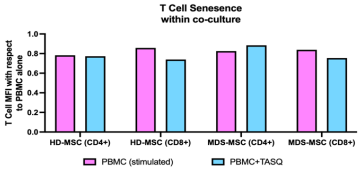

Figure 6 - MSC obtained from a healthy donor (N=1) and MDS donor (N=1) were co-cultured with stimulated PBMCs in triplicates with or without TASQ treatment. After 4 days of incubation, PBMCs from each sample were collected and FACS were performed for senescence markers. Specifically, DAPI-, CD45+ and CD4+ or CD8+ gating were performed and T cell senescence was measured according to global senescence marker CD28- - CD57+. Data are presented relative to stimulated PBMC samples from monoculture. (n=1)

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