Phenotypic Characterization of the Immune Landscape in the Bone Marrow of Patients with Acute Myeloid Leukemia (AML) Using MultiOmyx™ Hyperplexed Immunofluorescence Assay

#1455

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Introduction

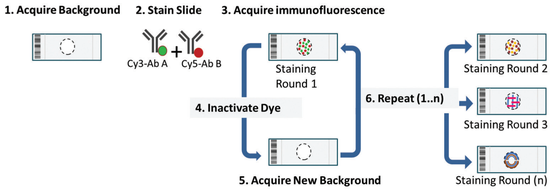
Background: Acute myeloid leukemia (AML) is a clinically and molecularly heterogeneous disorder. Bone marrow (BM) constitutes the home niche for leukemia cells in AML. Emerging data indicates that the BM microenvironment becomes immunosuppressive and plays a crucial role in cancer development and progression. Regulatory T cells (Tregs), tumor associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs) all contribute to immunologically permissive microenvironment for cancer cells. Based on phenotypical characteristics, MDSC can be further subdivided into granulocytic MDSC (G-MDSC, polymorphonuclear MDSC) and monocytic MDSC (M-MDSC). Although increasing evidence suggests that the immune system impacts the pathogenesis and prognosis in AML patients, only limited data has been published to comprehensively describe the immunological composition of AML BM microenvironment.

Methods: In this study, we aimed to perform comprehensive characterization of the immune cells in the BM of patients with AML. Using MultiOmyx hyperplexed immunofluorescence (IF) assay and proprietary imaging analysis, we studied BM tissues of AML patients and normal controls with a total of 13 markers essential in cancer immunology. The normal and AML BM FFPE sections were stained with CD34, Arginase1, CD11b, CD14, CD15, CD33, CD68, CD163, HLA-DR, CD3, CD4, CD8 and FOXP3.

Results: Overall, MultiOmyx 13-plex panel staining results revealed an immune suppression-skewed immune profile in AML BM in this study. We observed that both M-MDSCs and G-MDSCs accumulated within the tumor microenvironment (TME) in AML BM samples, with higher frequency of G-MDSCs over M- MDSCs. The data also revealed an abundant M2 macrophages present in the TME of the AML samples. The detection of both MDSCs and M2 macrophages in these samples supports the hypothesis that these cells contribute to the establishment of an immunosuppressive TME. Using the MultiOmyx proprietary algorithm, which takes into account the staining patterns, we quantified the counts and density of different immune cells in both AML patient and normal BM samples. There was a significantly higher frequency of M2 TAMs in AML than normal BM. Increased M-MDSC to G-MDSC ratio was also noted in patients with AML. Further, the spatial distance from the different subsets of immunosuppressive cells to CD34+ blasts was measured in AML samples using nearest neighbor analysis. The data indicated that G-MDSCs were spatially closer to CD34+ blasts in AML than M-MDSCs.

Conclusions: The direct assessment of immune phenotypes and their spatial relationship by MultiOmyx IF assay provides essential information in understanding the immune landscape in AML BM. Together, our data suggests that AML blasts may directly recruit immunosuppressive cells such as TAMs as one of the escape strategies. The potential for eradicating AML may lie in rational combinations of immunotherapies with strategies of the induction of anti-tumor immunity and the elimination or reprogramming of the immunosuppressive TME.

Assay Workflow & Panel Specifications



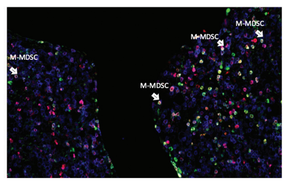


Figure 1. MultiOmyx multiplexing IF staining protocol. For each round of staining, conjugated fluorescent antibodies were applied to a 4 µm section, followed by image acquisition of stained slides. The dye was erased, enabling a subsequent round of staining with another pair of fluorescent antibodies. Proprietary cell segmentation algorithms generate unique IDs for every cell allowing them to be tracked through multiple rounds of staining.

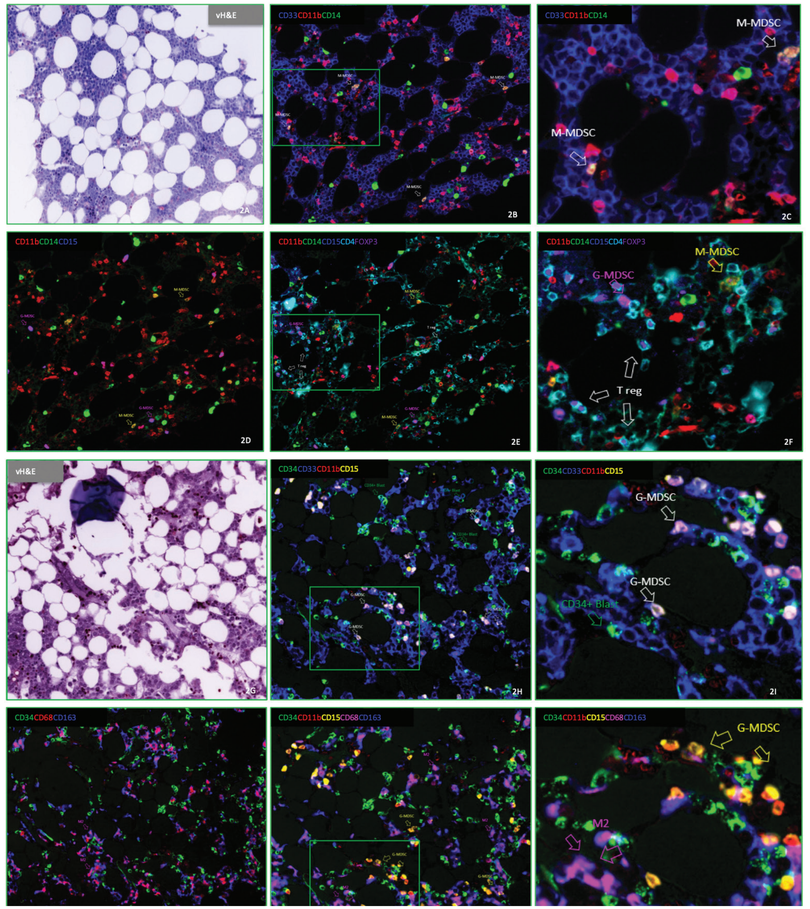
13-Marker Panel	
1	CD34
2	Arginase 1
3	CD11b
4	CD14
5	CD15
6	CD33
7	CD68
8	CD163
9	HLA-DR
10	CD3
11	CD4
12	CD8
13	FOXP3

Table 1. Multiplexing panel for MultiOmyx multiplexing staining used in the study.

Co-expression	Tumor Tissue Phenotype
CD3+CD4+	T helper cells
CD3+CD4+FoxP3+	T regulatory cells
CD3+CD8+	T cytotoxic cells
CD68+HLADR+CD163-	M1 TAMs
CD68+HLADR-CD163+	M2 TAMs
CD11b+CD33+	Myeloid cells
CD11b+CD33+HLA_DR-	MDSCs
CD11b+CD33+HLA_DR-CD14+	M-MDSCs
CD11b+CD33+HLA_DR-CD15+	G-MDSCs/TANs
CD11b+CD33+HLA_DR-CD15+ Arginase 1	Arginase 1 expressing G-MDSCs

Table 2. Phenotyping of human tumor-associated lymphocytes and myeloid cells in the study. Cell surface markers associated with cell subsets analyzed in the tumor samples. TAMs: tumor-associated macrophages. TANs: tumor-associated neutrophils MDSCs: myeloid-derived suppressor cells.

Characterization of Immune Suppressive Cells in AML



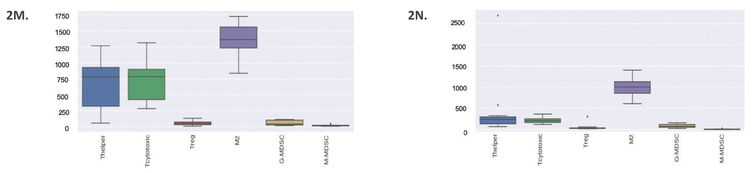


Figure 2. Characterization of Immuno-suppressive Cells using MultiOmyx Hyperplexed IF assay. 2A-2F are the representative images from AML sample #1. A, the vH&E image (simulated HE image from fluorescent signals) of the region of interest (ROI). B, representative color overlaid image for M-MDSC. M-MDSCs are the white cells positive for CD11b, CD33 and CD14. C, higher magnification of image B. D, representative images for MDSC. G-MDSCs (CD11b+CD15+CD14-) are magenta and M-MDSCs (CD11b+CD14+CD15-) are yellow. E, presence of Tregs in the same ROI with MDSCs. The white arrows indicate examples of Tregs (CD4+FOXP3+). F, higher magnification of image E. 2G-2L are the representative images from AML sample #2. G, the vH&E image of the ROI. H, representative images of G-MDSC. CD11b+CD33+CD15+ G-MDSCs are in white. CD34 stains the blasts. I, higher magnification of image H. J, representative M2 macrophages co-expressing CD68 and CD163 are seen in magenta. K, visualization of CD68+CD163+ M2 macrophages and CD11b+CD15+ G-MDSCs in the ROI. L, higher magnification of image K. 2M & 2N summarize slide level classification density results for different subtypes of immune suppressive cells in AML sample #1 & #2.

MultiOmyx Spatial Analytics – Nearest Neighbor Analysis

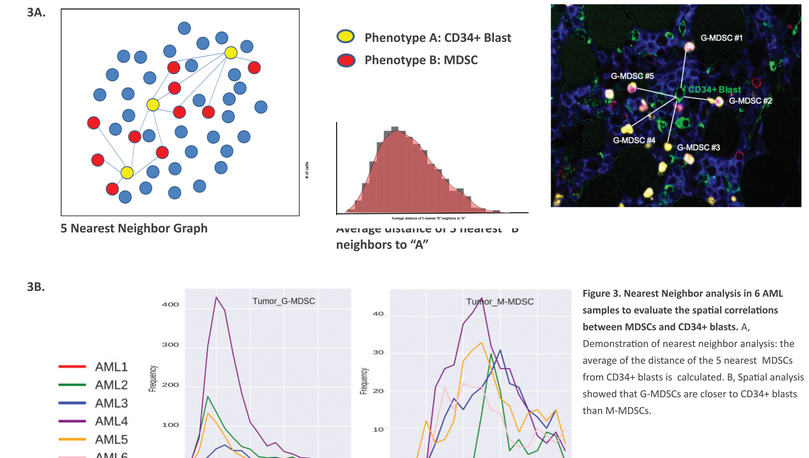


Figure 3. Nearest Neighbor analysis in 6 AML samples to evaluate the spatial correlations between MDSCs and CD34+ blasts. A, Demonstration of nearest neighbor analysis: the average of the distance of the 5 nearest MDSCs from CD34+ blasts is calculated. B, Spatial analysis showed that G-MDSCs are closer to CD34+ blasts than M-MDSCs.

AML vs. Normal Bone Marrow

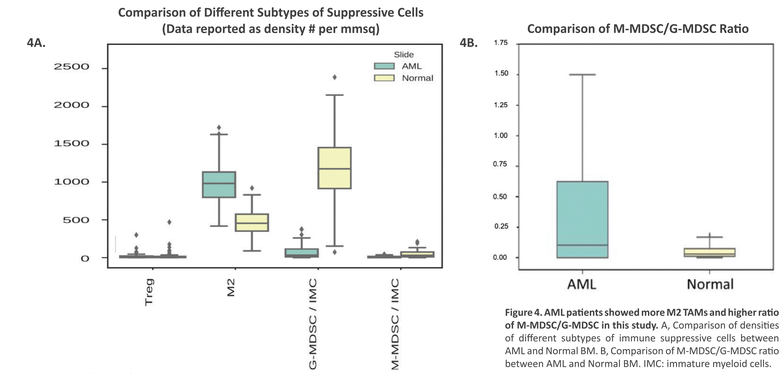


Figure 4. AML patients showed more M2 TAMs and higher ratio of M-MDSC/G-MDSC in this study. A, Comparison of densities of different subtypes of immune suppressive cells between AML and Normal BM. B, Comparison of M-MDSC/G-MDSC ratio between AML and Normal BM. IMC: immature myeloid cells.

Key Findings

- MultiOmyx 13-marker panel was used in this pilot study to characterize the immune landscape of FFPE samples from AML patients and healthy donors.
- Tregs, M2 TAMs and MDSCs were observed in AML FFPE samples.
- Increased M2 TAMs and M-MDSC/G-MDSC ratio were found in AML compared to normal BM in this study.
- In a spatial analysis of MDSCs and AML blasts, G-MDSCs were found to be in closer proximity to CD34+ blasts than M-MDSCs.
- T cells were observed in AML samples (data not shown) in this study. For next step, it will be interesting to use this panel combined with immune modulators to further understand T cell status within the TME for AML.

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