

INTRODUCTION

Multiple Myeloma (MM) is a plasma cell malignancy characterized by high genetic and microenvironmental heterogeneity. The emergence of extramedullary disease (EMD) represents an aggressive clinical phase linked to treatment resistance and poor prognosis. However, the mechanisms driving plasma cells to escape the bone marrow and infiltrate distant sites remain poorly understood.

MACSima™ Imaging Cyclic Staining (MICS) is a high-dimensional cyclic imaging technology that enables the simultaneous analysis of 56 distinct biomarkers on a single biological sample through repeated cycles of staining and signal erasure. The comparison of bone marrow and EMD samples is the method used to build a spatial map that highlights the structural differences supporting aggressive cell groups. This integrated framework is the key to seeing how certain cell networks help the disease grow and spread. By looking at these specific patterns, it becomes possible to understand the exact mechanisms that drive disease progression.

RESULTS

The application of MICS technology identified 14 distinct cell types in the bone marrow versus 9 in EMD, highlighting a significant reduction in cellular diversity. As illustrated in **Figure 1**, EMD lesions exhibit a severely disrupted architecture with a loss of stromal integrity and diminished cross-talk with regulatory immune cells. Within these sites, malignant plasma cells organize into dense clusters that lack canonical interactions with CD8⁺ T cells and dendritic cells. Spatial distance analyses (**Figure 3**) confirm this segregation, showing that EMD-resident plasma cells are markedly more distant from T-cells than their BM counterparts. As shown in **Figure 2**, this physical isolation coincides with an aggressive phenotype characterized by the upregulation of drug-resistance markers **BCL-2** and **EZH2**, alongside a significant reduction in **CD38** expression.

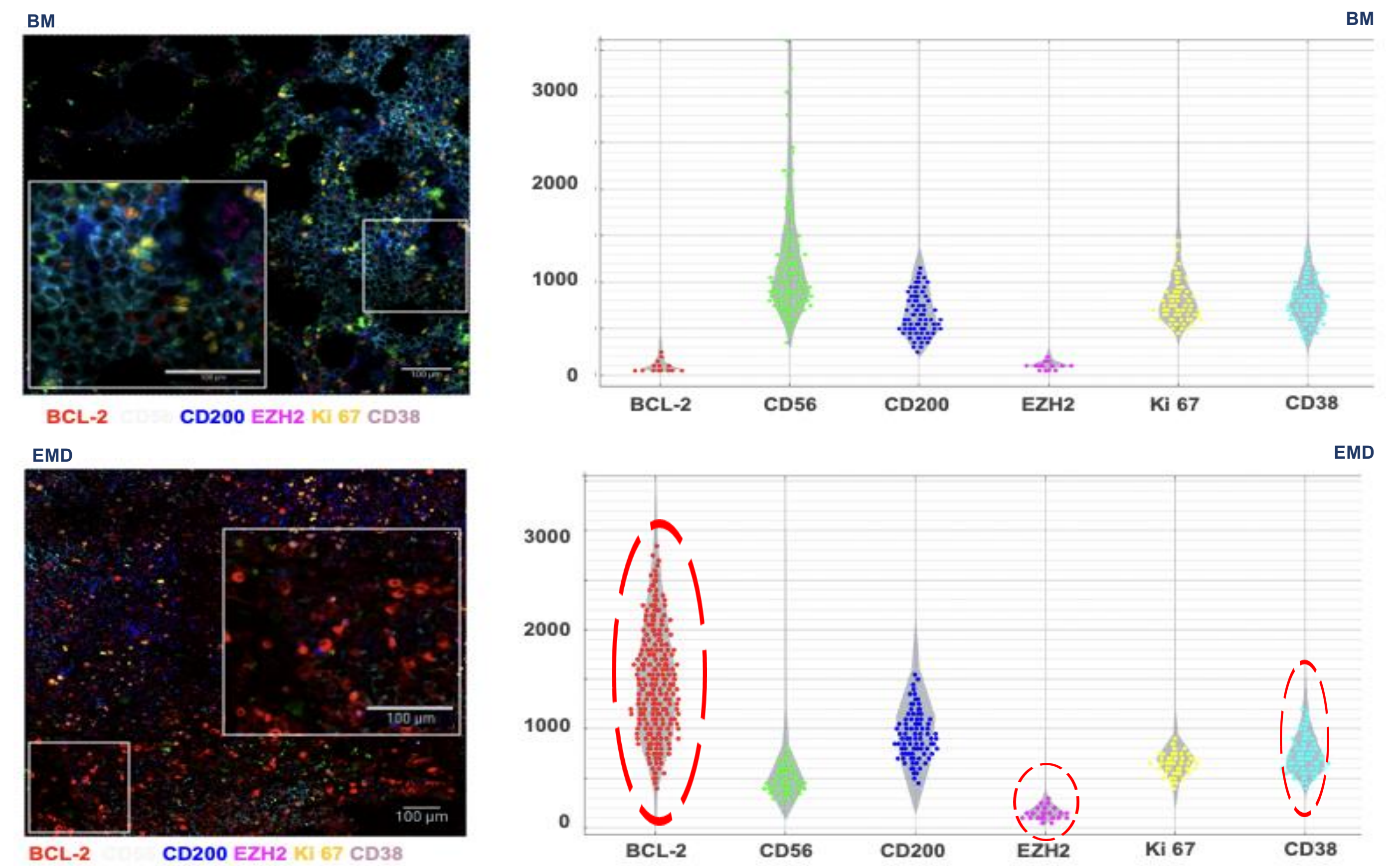


Figure 2. Analysis of biomarkers expression and spatial distribution comparing bone marrow (BM) and extramedullary disease (EMD).

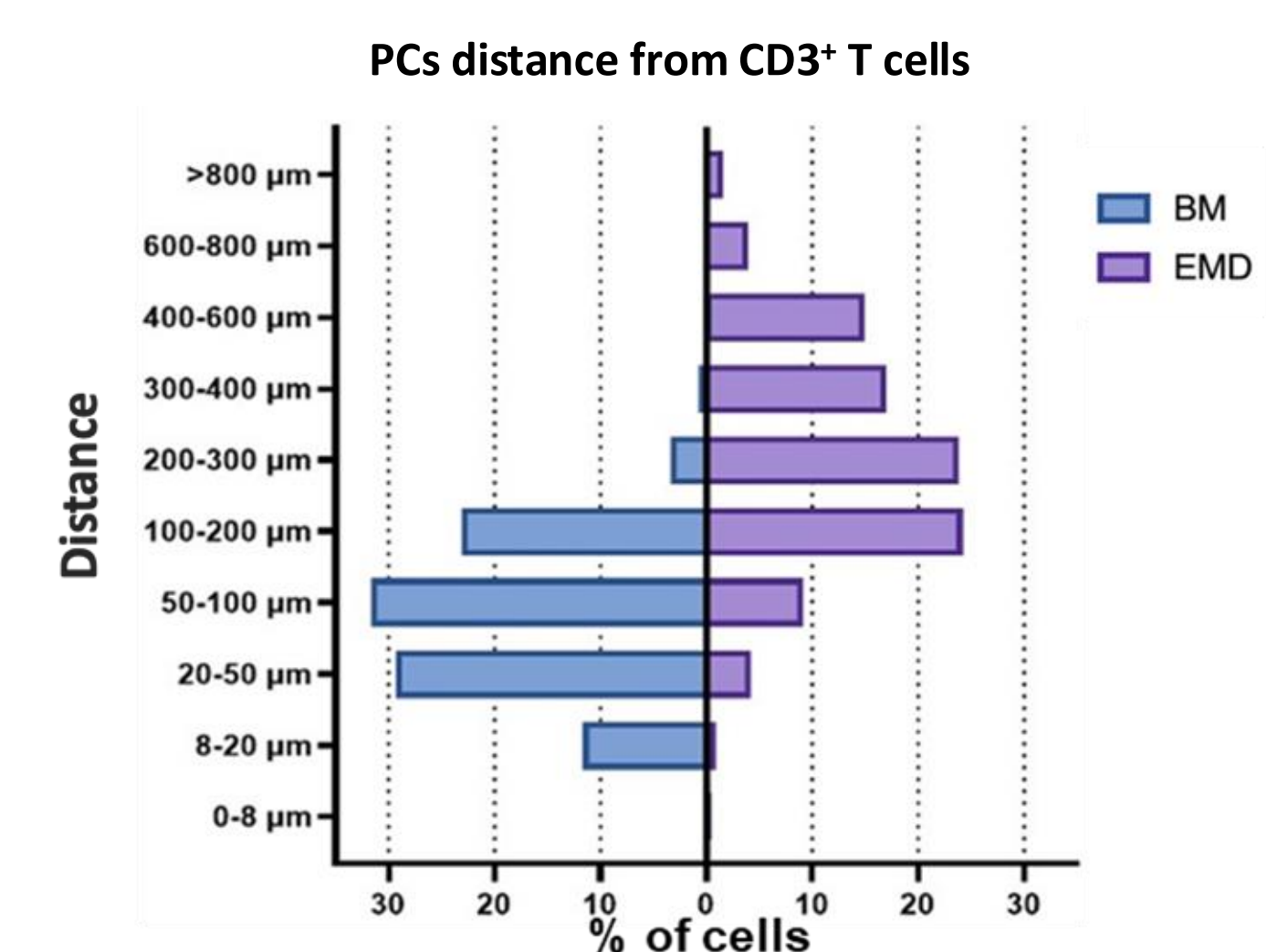


Figure 3. Butterfly graph comparing PCs distance from CD3⁺ T cells in representative MM#5 paired samples.

CONCLUSIONS

High-dimensional spatial imaging indicates that the progression to EMD is driven by a fundamental restructuring of the tumor microenvironment into protective niches. The formation of these immune-isolated environments, combined with a drug-resistant and CD38-low phenotype, provides a structural rationale for the limited efficacy of T-cell-mediated and CD38-targeted therapies in extramedullary settings. Consequently, addressing the specific architectural organization and cellular segregation within these lesions is essential for developing more effective precision medicine strategies in advanced Multiple Myeloma.

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Figure 1. Spatial profiling of bone marrow (BM) niche and extramedullary disease (EMD) in multiple myeloma.

