# Longitudinal Analysis of Multiple Myeloma: Therapeutic Response and Immune Microenvironment Dynamics in the Context of Tumor Heterogeneity


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## Introduction

Multiple myeloma (MM) is a hematological malignancy characterized by an expansion of malignant plasma cells in the bone marrow. For newly diagnosed MM (NDMM), standard three-drug treatment regimens—such as the combination of bortezomib, lenalidomide, and dexamethasone (VRd)—and the more recent inclusion of a fourth drug, anti-CD38 antibody immunotherapy, have significantly improved patient outcomes by targeting multiple mechanisms of tumorigenesis. This treatment approach typically involves several cycles of VRd induction therapy, followed by high dose melphalan (HDM) and autologous stem cell transplant (ASCT), and maintenance therapy. Treatment responses vary widely, with some patients requiring longer and more intense therapy and others exhibiting more rapid responses and longer relapse-free remission.This cycle of remission, maintenance, and eventual relapse persists despite advancements in treatment modalities, including immunotherapy, leaving MM incurable. This study characterizes the tumor state and immune microenvironment in MM patients across variable graded patient responses. Using a multimodal, longitudinal approach, we investigate immune and microenvironmental cellular changes during treatment in NDMM patients and in a second cohort of RRMM patients. For the NDMM cohort, longitudinal blood and bone marrow aspirate samples were collected at diagnosis, throughout induction therapy, following autologous stem cell transplant (ASCT), and at post-transplant intervals of 1 and 2 years.

Multi-omic profiling of these samples magnifies the individual heterogeneity between tumor and therapeutic response. The longitudinal aspect of the study allowed us to focus on individual-level longitudinal responses instead of cross-patient effects, enabling detailed insights into tumor and microenvironment dynamics. Using Olink proteomic analysis of plasma and bone marrow interstitial fluid (BMIF), and single-cell analyses (3' CITE-seq, scRNA-seq, flow cytometry) of peripheral blood mononuclear cells (PBMC) and bone marrow mononuclear cells (BMMC) we longitudinally characterized tumor, the immune microenvironment, and therapeutic responses. This revealed distinct immune states and pathways associated with therapeutic response, and tumor survival, highlighting potential targets and therapeutic strategies to improve patient outcomes.

## Study Design: Multi-modal approach to understand MM and the immune microenvironment

Figure 1: Multimodal longitudinal approach in Multiple Myeloma

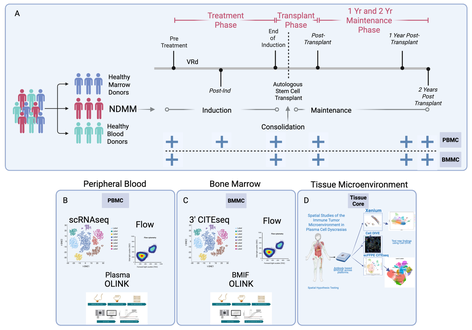


**Figure 1**: Multimodal approach to studying longitudinal plasma cell and immune dynamics in multiple myeloma (MM) patients during therapy.(A) Patient and healthy donor cohorts, NDMM treatment phases including induction, consolidation, autologous stem cell transplant, and maintenance.(B) Samples from 17 patients were collected longitudinally at key clinical timepoints throughout therapy and analyzed using proteomics, single-cell RNA sequencing, and flow cytometry. Samples from 10 healthy donors for BMMC and 50 Healthy PBMC donors as healthy controls (data not shown).

Figure 2: Longitudinal Single-Cell and Proteomic Analysis of Bone Marrow and Blood in Multiple Myeloma Patients Across Therapy

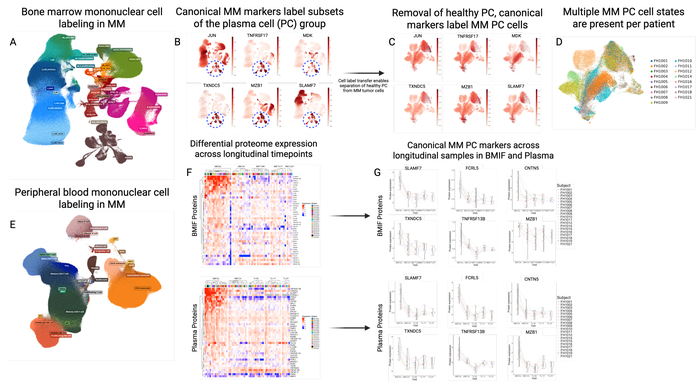


**Figure 2**: Single-cell transcriptomics and proteomics enable deep profiling of the tumor and immune microenvironment throughout therapy. (A, E) Bone marrow mononuclear cells (BMMCs) were analyzed using 3' CITE-seq, and peripheral blood mononuclear cells (PBMCs) were profiled using single-cell RNA sequencing. Cells were annotated using CellTypist and manual curation, incorporating reference datasets (Bandyopadhyay et al., 2024; Gong et al., 2024). In total, 840,089 BMMC cells and 1.7 million PBMCs were profiled and visualized using Uniform Manifold Approximation and Projection (UMAP), alongside a healthy BMMC dataset comprising 107,652 cells (not shown). (B–D, F, G) Canonical MM markers are visualized on UMAPs and highlight the MM plasma cell (PC) population. Heatmaps and line plots show differential expression of canonical protein biomarkers from OLINK across therapy.

## Single Cell and Proteomics analysis

Figure 3: Therapy-Associated Dynamics of Cyclin D–Defined Plasma Cell States and Differentiation in MM

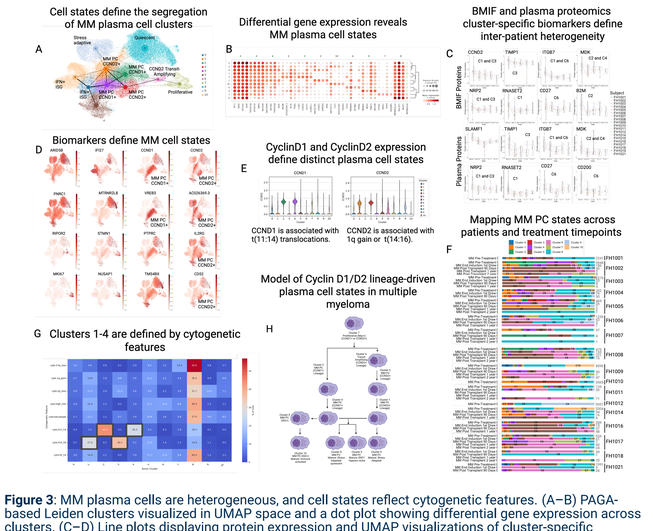


**Figure 3**: MM plasma cells are heterogeneous, and cell states reflect cytogenetic features. (A–B) PAGA-based Leiden clusters visualized in UMAP space and a dot plot showing differential gene expression across clusters. (C–D) Line plots displaying protein expression and UMAP visualizations of cluster-specific differentially expressed genes (DEGs). (F) Mapping of MM plasma cell clusters across therapeutic timepoints reveals that certain clusters persist into the maintenance phase in many patients. (G–H) A subset of clusters is associated with t(14;16) or t(11;14) cytogenetic abnormalities, suggesting a model in which some cell states are shared (Clusters 5/8/7), while others are distinct (Clusters 1, 3, 6 or 2, 4) and potentially lineage-driven.

## Results

Multiple myeloma (MM) develops through a series of well-characterized stages, beginning with precursor conditions such as monoclonal gammopathy of undetermined significance (MGUS), advancing to smoldering myeloma (SMM), and ultimately progressing to symptomatic MM. During this continuum, malignant plasma cells undergo ongoing genetic and transcriptional evolution, shifting between states of quiescence and proliferation, and eventually acquiring features that support tumor progression, dissemination, and relapse. This evolution is shaped not only by intrinsic factors, such as genomic instability, but also by extrinsic pressures including therapeutic interventions and immune surveillance, which can drive tumor plasticity and the selection of resistant subclones. Oncogenesis is typically initiated by primary genomic events such as hyperdiploidy or translocations involving the immunoglobulin heavy chain (IGH) locus—most commonly t(4;14), t(11;14), and t(14;16)—and is further shaped by the accumulation of secondary lesions, including subclonal mutations in key driver genes.

In this study, we employ a multimodal approach integrating single-cell transcriptomics, proteomics, and spatial analysis to characterize the mechanisms underpinning tumor evolution and cell state plasticity during therapy. By profiling both tumor and immune compartments across treatment stages, we aim to uncover how dynamic cellular states contribute to therapeutic response (Figure 1). Using proteomic and single-cell approaches (Figure 2), we have profiled over 2 million BMMC and PBMC cells collected from patients at diagnosis and across up to 2 years of therapy. Importantly, gene expression changes in markers commonly expressed at diagnosis in PBMC and BMMC samples are also found to be differentially expressed in proteomic data (Figure 2), underscoring the robustness of this system-wide approach.

In Figure 3, we examined tumor heterogeneity and found that tumors exist in multiple transcriptional states, many defined by the initiating genomic lesion. For example, Clusters 2 and 4 show distinct expression of *CCND1* (Cyclin D1), while Clusters 1 and 3 express *CCND2* (Cyclin D2)—genes commonly involved in t(11;14) and t(14;16) translocations, respectively. These genes define distinct tumor states and are not co-expressed within the same clusters. Despite these differences, several clusters are shared across tumors, including proliferative (Cluster 7), quiescent (Cluster 8), and tissue-embedded (Cluster 5) states. Notably, in patients with residual disease, tumor cells are frequently found in these shared clusters during the Maintenance phase (Figure 3F–H).

In Figure 4, we mapped tumor clusters (defined in Figure 3) onto spatial data and observed that tumor cells again occupy multiple cell states, corresponding to several Leiden-defined clusters (Figure 4A–B). We used Banksy to define cellular neighborhoods based on neighbor-aware gene expression. We found that proliferative tumor cells (Tumor 3; Cluster 7-like, Figure 3) and Tumor 2 (Cluster 6-like) are the least spatially connected. In contrast, Tumor 1 (Cluster 4-like) and Tumor 4 (Cluster 1-like) are highly embedded within spatial neighborhoods. Tumor 1 is primarily surrounded by macrophages, while Tumor 4 cells are encircled by myeloid/monocyte populations (Figure 4C–D). Moreover, Tumor 1 and Tumor 4 clusters often co-localize within the same spatial neighborhood and are found in proximity to cancer-associated fibroblasts (CAFs). These findings suggest that the spatial microenvironment may influence tumor cell state and behavior.

## Spatial Analysis of MM reveals different cell populations, neighborhoods and cell states

Figure 4: Mapping tumor cell states on the immune and tumor microenvironment

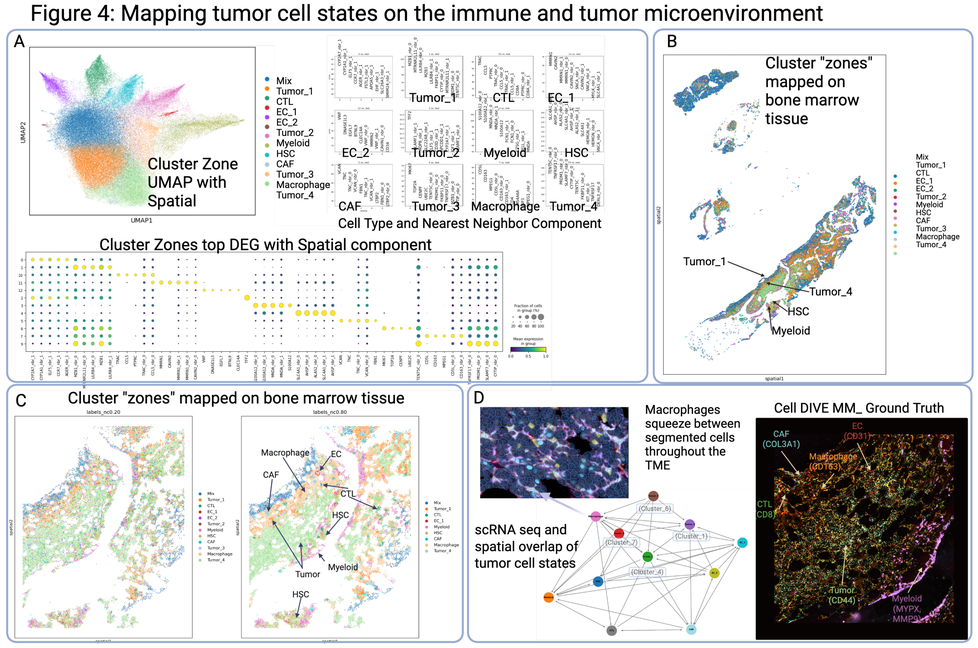


**Figure 4:** Xenium MM bone marrow data analyzed with Banksy reveals cell–cell interaction zones, validated by Cell DIVE.(A) Cell type identification and neighbor clustering define the immune and stromal microenvironment, as well as four distinct MM cell states.(B) Spatial mapping of identified cell clusters onto tissue sections.(C) Visualization of spatial interaction zones within the tumor microenvironment (TME).(D) Mechanistic validation using Cell DIVE and targeted antibodies. Multiplexed antibody labeling enables identification of cell–cell interactions not evident from spatial transcriptomic or probe-based approaches. *Top left image:* Macrophages (white/pink) weave between segmented cells and often contact multiple cell types—Ki67$^+$ (red), CD68$^+$ (yellow), and CD164$^+$/CD163$^+$ (pink/blue, macrophages). Macrophages form major hubs within spatial neighborhoods, particularly surrounding tumor cells, and mediate interactions not captured by transcriptomic profiling alone.

## Conclusions

Using a multimodal single-cell and spatial approach, we demonstrate that multiple myeloma tumor cells occupy diverse transcriptional states influenced by initiating genomic lesions, such as CCND1 and CCND2 translocations. Despite this heterogeneity, tumors converge on shared functional programs—proliferative, quiescent, and tissue-embedded—that persist across patients and treatment timepoints, including during residual disease. Spatial analysis reveals that these tumor states are differentially embedded within the microenvironment, with specific interactions between tumor cells, myeloid populations, and cancer-associated fibroblasts. Together, these findings highlight how both intrinsic tumor programs and extrinsic spatial context shape tumor cell plasticity and therapeutic resistance.

## References and Acknowledgements


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- **References:**


  - Bandyopadhyay S, Duffy MP, Ahn KJ, et al. Mapping the cellular biogeography of human bone marrow niches using single-cell transcriptomics and proteomic imaging. *Cell*. 2024;187(12):3120–3140.e29. doi:10.1016/j.cell.2024.04.008
  - Gong Q, Sharma M, Kuan EL, et al. Longitudinal Multi-omic Immune Profiling Reveals Age-Related Immune Cell Dynamics in Healthy Adults. bioRxiv [Preprint]. 2024 Sep 14:2024.09.10.612119. doi: 10.1101/2024.09.10.612119. PMID: 39314416; PMCID: PMC11419011.