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The Immune Microenvironment in Multiple Myeloma Progression at a Single-cell Level

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Multiple myeloma (MM) is a hematological malignancy of aberrant clonal plasma cells that reside within the bone marrow (BM).[1] The disease course differs from other BM malignancies essentially in 2 features. First, there are 2 precursor stages, monoclonal gammopathy of undetermined significance (MGUS) and smoldering MM (SMM), that can transition into MM over time.[2] Second, once clinical MM develops, the disease remains largely incurable, and despite significant therapeutic improvements, relapse and refractoriness usually cannot be prevented. The interactions between MM cells and the BM microenvironment (BM-ME) are an area of particularly intense research, as tumor evasion and suppression of the host immune system constellate main factors of MM progression.[3] Single-cell sequencing technologies that have emerged over the last years have the potential to significantly advance the field because they enable the evaluation of alterations in cell numbers and states as well as interactions between MM cells and the BM-ME. Recent studies have applied single-cell techniques at different precursor and MM stages to determine the comprehensive changes in the BM-ME and to identify mechanisms that foster oncogenesis.[4–11] This article briefly summarizes these studies and proposes how the dissection of the BM-ME on a single-cell level can improve our understanding of MM pathogenesis, thereby advancing prognostication and the therapeutic landscape. We would like to emphasize that single-cell analyses are a rapidly expanding field and the number of published studies investigating the BM-ME in MM and its precursors is likely to grow exponentially in the next few years given the increased interest in and access to single-cell technologies. Thus, this perspective does not cover all available articles.

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THE IMMUNE MICROENVIRONMENT IN THE EARLY EVOLUTION OF MYELOMA

Progression from precursor stages to clinical MM is highly heterogeneous, with some patients progressing quickly, while the others remain stable for decades.[2] Treatment is hence not justified solely on the premise of a precursor diagnosis. Hence, recent research has focused on identifying SMM patients, who would benefit from early therapeutic interventions. Current risk classifiers are mainly based on clinical parameters and tumor aberrations (translocation t[4;14], t[14;16], gain1q and/or del13q), yet are only able to capture up to ~60% of patients who progress to MM within 2 years.[12] Further molecular characterization of the BM-ME could improve the accuracy of prognostication and also pave the way for better therapeutic avenues. In that regard, Zavidij et al[8] performed single-cell RNA sequencing (scRNA-seq) of the immune-ME from 5 MGUS, 11 SMM and 7 MM patients compared with 9 healthy individuals. The authors show that substantial alterations of the immune-ME are already present at the MGUS stage. These include increased populations of natural killer (NK) cells, T cells, particularly Tregs, and nonclassical CD16+ monocytes. CD14+ monocytes showed dysregulated expression of major histocompatibility complex (MHC) type II genes, which resulted in T-cell suppression in in vitro cultures. The progression from MGUS to SMM was associated with the loss of granzyme K+ memory cytotoxic T cells leading to reduced immunosurveillance in in vivo models. Symptomatic MM was characterized by an increased INF-alpha response across all immune cell types, which has been shown to promote immunosuppression, favoring expansion of MM cells. Similarly, using scRNA-seq and mass cytometry, Bailur et al[11] showed that precursor stages already harbor BM-ME alterations compared with healthy donors, including early changes in NK and myeloid cells as well as increased terminal effector differentiation and enrichment of stem-like T cells in MGUS. Another scRNA-seq study with 8 MGUS, 7 SMM, and 10 MM patients showed substantial alterations in the immune-ME of MM compared with its precursor stages.[5] Symptomatic MM was enriched for CD14+ and CD16+ monocyte populations, memory B and CD8 effector cells compared with early stages with a transitional decrease of CD4-positive T cells and MAIT cells. However, there was no differential expression of exhaustion markers such as *PD1*, *TIGIT*, and *LAG-3* when comparing stages. Importantly, cell proportions were heterogeneous even across the same disease stage, emphasizing the need for larger datasets and also underscoring the existence of an individual immune phenotype, which is likely influenced by other factors than the aberrant plasma cells.

THE IMMUNE MICROENVIRONMENT DURING MYELOMA THERAPY AND IN RELAPSING DISEASE

Established therapeutic strategies yield high complete remission rates, but disease relapse will ensue in the vast majority of patients. Yet, the disease course remains very heterogeneous with some patients relapsing within months of treatment initiation and others experiencing deep remission for >10 years. This observation has led to the hypothesis that MM cells can enter a dormant state with regrowth over time when conditions become permissive, particularly when immune surveillance falters.[13] Although many MM therapies try to stimulate the immune-ME to fight the disease, it has been also shown that therapy causes immunosuppression with ensuing cytopenias and hypogammaglobulinemia, emphasizing that the balance of a stimulated or dysfunctioning immune-ME is very delicate. Deciphering the alterations within the immune-ME during therapy will hence be challenging.

Tirier et al[6] investigated 20 relapsed/refractory MM patients prior and post salvage therapy and compared them to 8 healthy donors. Applying scRNA-seq to the immune-ME and plasma cells, the authors analyzed both compositional changes and alterations in cell–cell interactions. In this population, which was heavily enriched for gain1q, the investigators found depletion of CD4+/CD8+ naïve and CD4+ memory cells with enrichment of CD14+/CD16+ monocytes, effector T-cell populations (CD8+ memory and cytotoxic cells) as well as gamma/delta T cells with an increase of inflammatory markers in the BM that were primarily secreted by CD14+ monocytes and MM cells. In a similar attempt to understand co-evolution of MM cells and their immune-ME, Liu et al[4] described longitudinal alterations during disease progression in 14 MM patients. They report that genetic alterations tend to be associated with distinct immune cells clusters, for example, patients with translocation t(11;14) showed separate T-cell clusters with upregulation of lysine methyltransferase genes *KMT2A* and *KMT2C* in CD8+ T cells.

Other studies addressed the role of the immune-ME in early relapse patients. Pilcher et al[7] performed scRNA-seq on the immune-ME in patients with rapidly progressing disease (<18 months) compared with those without progression within 4 years of follow-up. Early relapse patients had significantly higher numbers of exhausted CD8+ T cells (GZMK+ and TIGIT) with decreased expression of cytotoxic markers (PRF1, GZMB, and GNLY). Similar to the study by Tirier et al, there were also alterations within the monocyte/macrophage compartment with an increase of M2 macrophages in rapid progressors. Yao et al combined scRNA-seq with an additional capture of surface markers (Cellular Indexing of Transcriptomes and Epitopes by Sequencing, CITE-Seq) and the mass cytometry approach cytometry by time of flight (CyTOF) to elucidate the biology of early relapse.[9] Stratifying 18 MM patients by international staging system (ISS) and by time to progression (<6 months for rapid progressors or 6 months to 5 years for slow progressors), they found aggressive disease (ISS 3) to be associated with a decrease in CD4+ T cells and the CD4/CD8 ratio.

Last but not least, recent studies investigated alterations of the BM-ME during immunotherapies, as those heavily rely on a functional immune system for optimal efficacy. Adams III et al determined the effect of the CD38-antibody Daratumumab on immune cells in the BM and peripheral blood (PB) in relapsed MM patients.[10] Daratumumab depleted NK cells and reduced the amount of CD38+ basophils. As seen in the previous studies, immune profiling differed between responders and nonresponders with higher granzyme B expression in CD8+ T cells of responders, suggesting treatment induced T-cell activation with increased killing capacity. Friedrich et al determined the immunological single-cell mechanisms of resistance to bispecific BCMAxCD3 T-cell engager (TCE) therapy, a novel immunotherapy leading to unprecedented response rates in refractory MM.[14] They show that TCEs lead to clonal expansion of immune cell subsets, particularly effector CD8+ T cells and that primary or acquired failure to TCE therapy is associated with exhaustion of CD8+ T cells. Importantly, treatment failure or loss of response was not solely associated with immune-ME alterations but also MM intrinsic adaptations, such as loss of the target epitope and MHC class I protein.

Taken together, current research is rapidly progressing to identifying alterations within the immune-ME of particularly aggressive disease, as these patients are in most need of novel therapeutic approaches. Single-cell sequencing studies so far appear to uniformly show dysregulation of T cells, particularly cytotoxic/effector T cells with increase of exhaustion markers in progressing patients. Furthermore, there appear to be alterations leading to a dysfunctional state in the monocytes/macrophages compartment in more aggressive MM.

CHALLENGES AND FUTURE PERSPECTIVES

Single-cell techniques have the potential to comprehensively dissect disease mechanisms within the immune-ME and their relationship with MM cells, thereby offering a path to truly individualized medicine leading to improved prognostic measure and therapeutic avenues. Challenges remain in unifying cell subset annotations to better compare studies. The encountered heterogeneity between patients could be accounted for by increasing sample sizes and by stratifying patients based on the parameters such as age, genetic profiles of MM, and treatment received. Age has shown to have a significant impact on immune function and is manifested at multiple levels including reduced production of B and T cells and diminished function of mature lymphocytes.[15] It is hence not surprising that increased age has repeatedly shown to be an adverse risk factor for clinical outcome in MM, which is likely not only due to the higher prevalence of comorbidities in this population.[16] The notion that molecular subgroups of MM can differ in the surrounding BM-ME has been reported long before the onset of single-cell technologies. For example, patients of the low bone disease molecular subgroup barely show osteolytic lesions, suggesting that this subset spares the stimulation of osteoclasts and the resulting bone resorption.[17] The recently discovered associations of an immune-ME signature and +1q MM and the distinct clustering of immune cells in patients with translocation t(11;14) further corroborate the hypothesis that the MM genotype and phenotype influences its surrounding or vice versa. Hence, analyzing MM in its BM-ME on a single-cell level will offer the unique opportunity to associate alterations in the ME to intrinsic MM cell features, which could revolutionize our understanding of MM biology.

Treatment has a significant effect on the immune-ME, and can lead to alterations in cell populations and expression patterns, which will need to be taken into account when analyzing single-cell data.[10,18,19] These alterations are associated with immune cell cytotoxicity, exhaustion, and senescence, which can influence the further disease course.[20] Conversely, comprehensive immune profiling before therapy could determine which treatment modalities would yield best responses. The use of single-cell techniques before and during therapy would enable us to dissect mechanisms of response versus resistance and could tailor therapeutic approaches based on the patients' individualized immune profile.

Additional challenges to single-cell technologies are based on the current limitations of these techniques and are unlikely to be overcome by merely increasing the number of samples. This includes the limited number of analyzed cells due to the high costs. As an alternative or complementary approach, multidimensional flow cytometry allows for a cost-effective assessment of immune-phenotypes in the BM-ME and yields prognostic information in MM and its precursor stages.[19,21,22] A particular hindrance to single-cell technologies is the lack
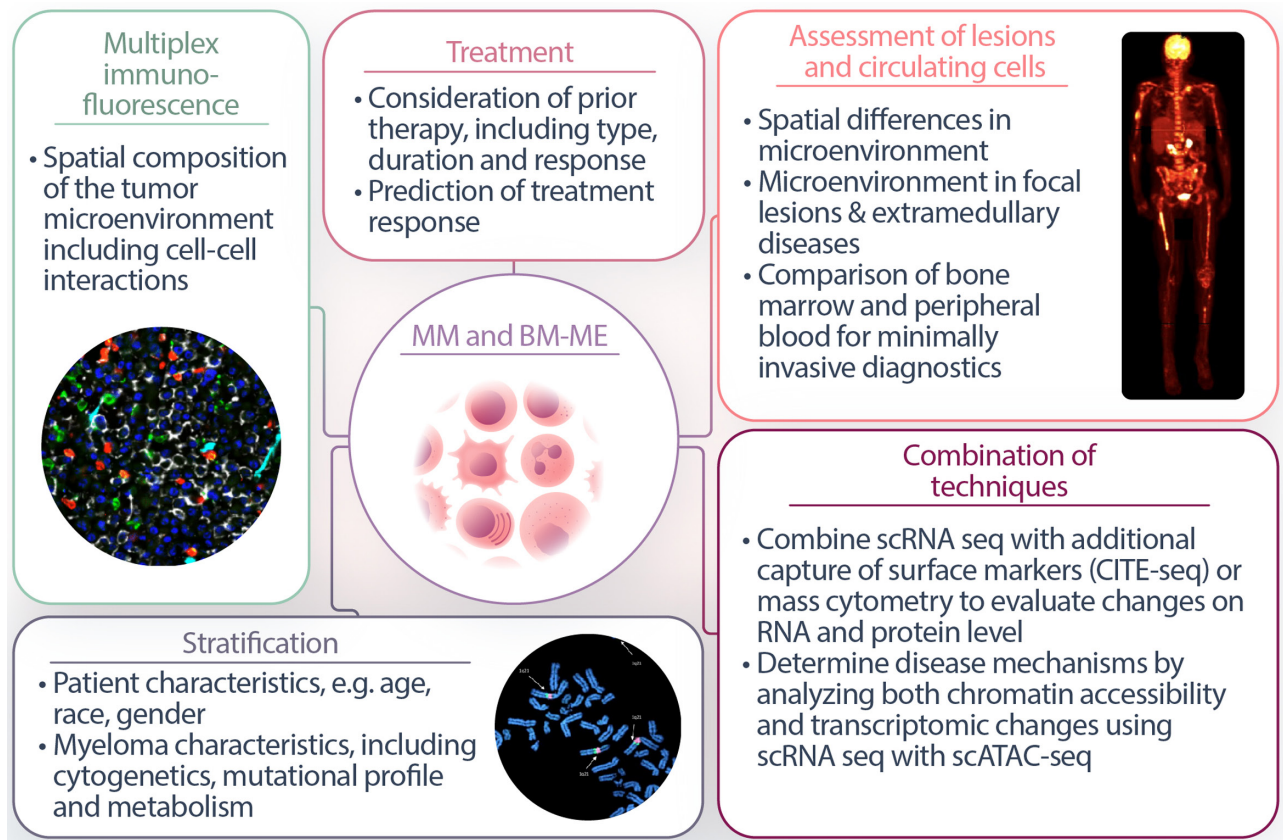
Figure 1. Summary of the anticipated future comprehensive analysis of myeloma and its microenvironment at a single-cell level.

of spatial resolution as they are performed on BM aspirates or PB. Although the current algorithms can predict interactions between cell populations based on the surface markers, the BM-ME architecture remains elusive. Furthermore, aspirates contain very small numbers of adherent cells, such as stromal cells, osteoblasts, and osteoclasts. Hence, to determine the structure and composition of the BM and its relationship with MM in great detail, it will be important to combine single-cell technologies with multiplex immunohistochemistry studies.[23]

Of note, BM-ME single-cell data are usually derived from a random BM site and might not be representative of the whole disease process. This is particularly important for MM, as the vast majority of patients present with focal lesions where genetic profiles of MM cells have been shown to be distinct from the random BM site.[24,25] First reports suggest that this also holds true for the BM-ME at these sites.[26,27] There is growing awareness that MM cell heterogeneity is, to at least some degree, reflected by circulating tumor cells, which are thought to originate from all affected MM sites and have prognostic impact.[22] Thus, paired profiling of the BM and PB could unravel alterations that are not evident at the random BM site. A summary of the anticipated comprehensive analysis of MM and its BM-ME in the future is presented in Figure 1.

In conclusion, single-cell technologies have already influenced the MM field by identifying mechanisms underlying treatment resistance and they have the potential to further advance our current understanding of risk classification, prognostication, and individualized treatment approaches. While high costs currently limit their application, it is anticipated that technical advances and broader use will significantly reduce the costs and allow for larger sample collections. Hence, comprehensive and longitudinal profiling of the immune-ME using multiomic single-cell approaches with integration of the spatial BM architecture and genomic profiling of MM cells could soon be a reality for our MM patients and will eventually lead to improved clinical outcomes and cure.

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