

Multiple myeloma - Section 13

Bone marrow niche in multiple myeloma and its precursor states

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Take home messages

- Bone marrow microenvironment in multiple myeloma provides a growth advantage for the malignant clones.
- T cells expressing the inhibitory molecule T cell immunoglobulin and ITIM domain and myeloid-derived suppressor cells facilitate myeloma progression.
- In Smoldering myeloma, early Tregs activation and defective antigen presentation are observed in the bone marrow microenvironment.

Introduction

Multiple myeloma (MM) is an incurable plasma cell malignancy with significant interpatient heterogeneity, accounting for 10% of all hematological malignancies.¹ Despite the introduction of immunomodulatory drugs and proteasome inhibitors, many patients with high-risk features still have modest progression-free survival rates and poor overall survival.

MM usually progresses from asymptomatic precursor stages, namely monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM).² The risk of progression from MGUS and SMM to overt myeloma constitutes a continuum, whereby certain patients progress rather quickly, while others never do. Several prognostic models have been developed to capture that variability and identify patients with high-risk MGUS and SMM who are likely to progress rapidly to overt MM. However, the majority of the biomarkers currently in use only measure tumor burden and disease activity or describe cytogenetics, which cannot fully account for the variability

observed in risk of progression. Instead, the tumor microenvironment in the bone marrow niche should be interrogated in parallel, to better understand disease progression and resistance, to develop more tailored therapies, and to improve patient outcome.

Current state of the art

Bone marrow microenvironment in MM

Out of the microenvironment's cellular compartment, T cells are perhaps the most well studied in relation to cancer evolution and progression. Myeloma cells can harbor a number of genetic alterations that distinguish them from normal cells at the protein level, thereby inducing tumor-reactive T cell responses. However, those responses are quickly muted by tumor cells, which curtail T cell effector functions via a spectrum of immunosuppressive mechanisms. These mechanisms include the high expression of inhibitory molecules, such as programmed cell death 1 (PD-1), lymphocyte-activation gene 3 (Lag-3), cytotoxic T lymphocyte antigen-4 (CTLA-4), and T cell immunoglobulin and ITIM domain (TIGIT). Previous studies demonstrated that PD-L1 is expressed on MM plasma cells and especially in relapsed disease,³ while PD-1 expression was reported to be upregulated in NK and T cells of MM patients,^{4,5} whose T cells reportedly exhibit a senescent phenotype.⁶ Nonetheless, clinical results of single-agent PD-1 inhibitor in relapsed myeloma were underwhelming,⁷ and combination therapy with other antimyeloma drugs has been halted due to serious toxicities in a group of patients.⁸ However, recent studies demonstrated that myeloma burden in Vκ*MYC mice correlates with the percentage of CD8+ TIGIT+ cells and that TIGIT is the most expressed checkpoint molecule in diseased mice. More importantly though, myeloma grew more slowly in TIGIT-null mice, and tumor burden was reduced with anti-TIGIT antibody treatment, raising the possibility of new immunotherapy options for MM patients.⁹ What is more, regulatory T cells (Tregs), that are normally involved

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in immune self-tolerance, can be coopted by cancer cells to aid in immune suppression and evasion. Indeed, there has been growing evidence supporting the role of Tregs in MM progression.^{9,10} Most importantly though, CD38-expressing Tregs were described as suppressive modulators of antitumor immune responses in MM, and CD38 targeting was shown to inhibit immunosuppression in *ex vivo* cocultures.¹¹ These results suggest a potential for anti-CD38 monoclonal antibody treatment to regulate the immune compartment of the bone marrow niche, as well as the tumor.

Furthermore, in recent years, another microenvironmental cell type, myeloid-derived suppressor cells (MDSCs), has been implicated in cancer-related immunosuppression. In MM, CD14⁺HLA-DR^{-low} monocytic MDSCs (M-MDSCs) are significantly increased in patients with newly diagnosed and relapsed disease. A recent study showed that the IL-18 induces MDSC activity, which accelerates MM progression by suppressing T cell response, while IL-18-deficient Vk*MYC mice were significantly protected from MM progression in a CD8⁺T cell-dependent manner.¹²

Collectively, these results underline the importance of a protective bone marrow niche even in the presence of overt disease and suggest that bone marrow niche normalization might be necessary for disease eradication. A list of current clinical trials of immunotherapy in MM can be found in (Table 1).

Bone marrow niche in MGUS and SMM

Using single-cell RNA sequencing, our group recently showed that the tumor immune microenvironment can already be altered at as early a stage as MGUS.¹³ Specifically, an enrichment in NK cells and nonclassical monocytes was observed, together with a decrease in plasmacytoid dendritic cells. On top of that the

alterations extended beyond the mere composition of the immune bone marrow niche into functional dysregulation. Exemplifying that quality, HLA expression in CD14⁺ monocytes was found to be upregulated, although the corresponding surface protein levels were shown to be reduced, suggesting an antigen-presentation defect is established early in disease progression. Moreover, coculture experiments showed that MM cells are indeed responsible for this dysregulation, favorably shaping the immune microenvironment at the precursor stage. These results suggest that parallel characterization of the immune microenvironment could improve risk stratification and thus patient management.

In another study by our group, Tregs expansion was observed in bone marrow samples of patients with SMM, as well as Vk*MyC mice with early-stage disease, suggesting early establishment of tumor-related immunosuppression in MM disease progression. More importantly, though, it was shown that *in vivo* Treg expansion negatively impacted survival, whereas mice survived longer in the absence of bone marrow Tregs, which designates Treg expansion as a potential therapeutic target for prevention of progression in MM. Indeed, it was shown that Treg expansion is driven by MM cells through secretion of type 1 IFN, which is reversible by anti-IFNAR1 antibodies.

Notwithstanding, in the era of single-cell RNA sequencing and immunotherapy, it is important to note that the tumor microenvironment comprises more than just immune cells. In fact, it consists of multiple other cell types that could be coopted by MM cells to promote progression, as well as the extracellular matrix (ECM), a noncellular component comprising a collection of proteins with an established role in various malignancies.¹⁴ In a recent study of the bone marrow ECM by our group, performed on bone marrow samples from patients with MGUS, newly diagnosed and relapsed MM, 2 ECM proteins, ANXA2 and LGALS1, were shown to only appear in the niche at progression to myeloma.¹⁵ These results were in concordance with tumor RNA expression data that showed an upregulation of the corresponding transcripts, indicating once again that the tumor itself is actively shaping its microenvironment. Intriguingly, though, we also observed proteins that were only altered at the MGUS stage of disease, suggesting that ECM alterations can take place early in disease progression, just like alterations of the immune microenvironment.

Table 1

Current Immunotherapy Trials Targeting the Immune Microenvironment in Multiple Myeloma

Clinical Trial No.	Immunotherapy Agent ^a	Phase
NCT03376477	GVAX (GM-CSF-transfected tumor cells)	2
NCT03266692	ACTR087 (ACTR-transfected T-cells)/SEA-BCMA	1
NCT03782064	Nivolumab (PD-1)/DC-MM fusion vaccine	2
NCT03634800	Nivolumab (PD-1)	2
NCT02906332	Pembrolizumab (PD-1)	2
NCT02077959	Pidilizumab (PD-1, DLL-1)	1/2
NCT03605719	Nivolumab (PD-1)/Reovirus	1
NCT01995708	Dendritic cells (CT7, MAGE-A3, WT1)	1
NCT03457142	Abatacept (CTLA-4)	2
NCT03464916	Anti-CD38 CAR-T cells	1
NCT03651128	Anti-BCMA CAR-T cells	3
NCT03549442	Anti-BCMA CAR-T cells	1
NCT03602612	Anti-BCMA CAR-T cells	1
NCT03591614	Dendritic cells (DKK1)	1
NCT01067287	Pidilizumab (PD-1, DLL-1)/DC-MM fusion vaccine	2
NCT03582033	Anti-BCMA CAR-T cells	1
NCT03070327	Anti-BCMA CAR-T cells	1
NCT02215967	Anti-BCMA CAR-T cells	1
NCT03761108	Anti-BCMA/anti-CD3 bispecific antibody	1/2
NCT03288493	Anti-BCMA CAR-T cells	1
NCT03548207	Anti-BCMA CAR-T cells	1/2
NCT03269136	Anti-BCMA/anti-CD3 bispecific antibody	1
NCT03486067	Anti-BCMA/anti-CD3 bispecific antibody	1
NCT03318861	Anti-BCMA CAR-T cells	1
NCT02064387	Anti-BCMA CAR-T cells	1
NCT03145181	Anti-BCMA/anti-CD3 bispecific antibody	1
NCT03340883	Anti-BCMA CAR-T cells	1/2

PD = programmed cell death 1.

^a Only the immunotherapy agents tested are listed here; refer to ClinicalTrials.gov for full regimen.

Future perspectives

An important implication of these discoveries on the precursor-stage microenvironment is that MM disease progression cannot be fully understood or predicted outside the context of the bone marrow niche. But more importantly, MM disease progression might be amenable to interception by early therapeutic targeting of bone marrow niche components.

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