Targeting Tumour-Associated Macrophages for Multiple Myeloma Therapy

Alexander Wong
University of Toronto Schools
Sun Life Gene Medical Science Institute Cancer Research Program Student, Toronto, Ontario, Canada

Abstract:
Multiple myeloma (MM) is a hematologic malignancy characterized by the accumulation of malignant plasma cells in the bone marrow. Tumour-associated macrophages (TAMs) play a crucial role in the tumour microenvironment of MM, contributing to tumour progression, immune evasion, and drug resistance. Recent studies have highlighted the potential of targeting TAMs as a therapeutic strategy for MM, with various therapies being developed. This paper aims to explore the therapeutic potential of various TAMs in relation to MM as a method for treatment. To do so, various scientific studies have been analyzed, utilizing the data for various antibody therapies and their results to explore their potential as viable treatments. Ultimately, recent developments have shown that these therapies are indeed effective and can inhibit the progression of MM, paving the way for combating the proliferation of MM cells.

Keywords: tumour-associated macrophage; therapies; multiple myeloma; microenvironment; angiogenesis.

Introduction:

Multiple myeloma (MM) is a cancer of the plasma cells within the bone marrow. Initially, patients with MM contain cancerous cells within their bone marrow tissue that, over time, grow and proliferate until the healthy blood cells are overcrowded. MM cells continuously produce a special type of antibody known as monoclonal proteins (M proteins), but the human body cannot use them, which ultimately causes severe damage (Mayo Clinic, 2023). Symptoms of MM include, but are not limited to, bone pain, anemia, renal dysfunction, and hypercalcemia. Additionally, the pathogenesis of MM involves complex genetic and molecular alterations, including dysregulation of signalling pathways that govern cell growth, survival, and apoptosis (Mayo Clinic, 2023). However, in recent years, despite the introduction of many novel drug therapies, the disease has proven to be highly drug-resistant. In MM, the tumour microenvironment (TME) within the bone marrow niche consists of dendritic cells, T-cytotoxic, T-helper, reactive B-lymphoid cells, and macrophages (Cencini et al., 2023).

Macrophages are specialized white blood cells crucial for the human body's immune function. They originate from blood monocytes that differentiate in various types of tissue and are often involved in the detection, phagocytosis, and destruction of bacteria and other harmful organisms. In addition, they can also present antigens to T cells and initiate inflammation by releasing molecules, known as cytokines, that activate other cells. Within the body, macrophages migrate to and circulate within almost every tissue, patrolling for pathogens and eliminating dead cells. To do so, macrophages produce reactive oxygen compounds, such as nitric oxide, that kill phagocytosed bacteria (Saldana, n.d.).

The major functions of macrophages include phagocytosis, antigen presentation, and cytokine production. Phagocytosis is a primarily eukaryotic process that occurs in the plasma membrane of a cell, where it is directed by cytoskeletal filaments to form pseudopodia that act to engulf a particle and bring it into the cell from the extracellular matrix (Deshpande & Wadhwa, 2023). Antigen presentation is the process of displaying unique components of antigenic fragments—epitopes—to the immune cells that contain the corresponding antigen receptors (Nesmiyanov, 2022). Cytokine production is the process by which macrophages secrete cytokines such as tumour necrosis factor (TNF), IL-1, IL-6, IL-8, and IL-12 (Duque & Descoteaux, 2014). Moreover, macrophages additionally secrete a variety of cytokines in response to activation.

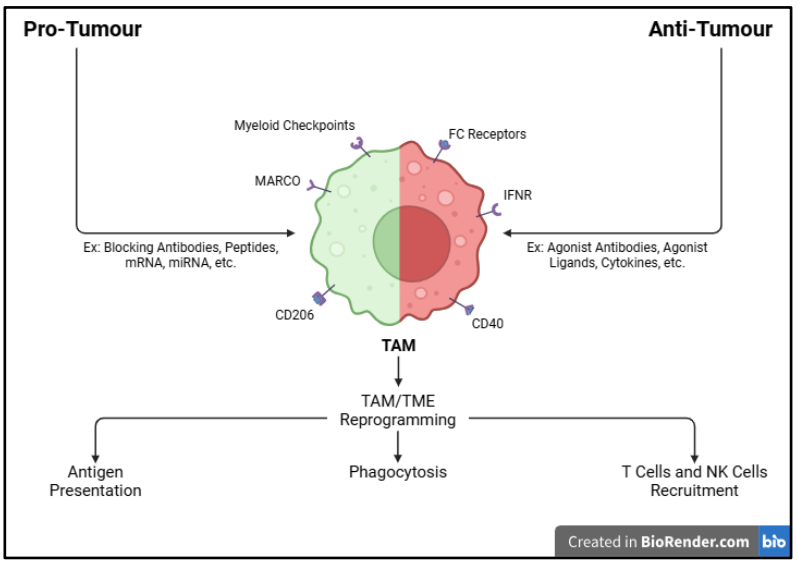

Figure 1. Image of the macrophage reprogramming process and the activation of innate and adaptive immune responses created using BioRender.com.

Macrophages also play an important role in immune modulation and tissue homeostasis, being able to polarize into different functional states (M1 and M2) based on the various signals in their environment. M1 macrophages are pro-inflammatory and involved in defence against infections, while M2 macrophages are anti-inflammatory and contribute to tissue repair and homeostasis (Yunna et al., 2020). Specifically, M1 macrophages are classically activated, either by IFN-γ or lipopolysaccharide (LPS), and produce phagocytize microbes, nitric oxide, or reactive oxygen intermediates to combat bacteria and viruses. M2 macrophages, on the other hand, are alternatively activated by exposure to certain cytokines, such as IL-4, IL-10, or IL-13, and will produce either polyamine to induce proliferation or proline to induce collagen production. Additionally, M2 macrophages can polarize into various subtypes including M2a, M2c, and M2d depending on their activation state and function. Nonetheless, they all often play a role in maintaining the balance and normal functioning of tissues, being involved in tissue repair, regeneration, and the removal of damaged cells (Lodge, 2020).

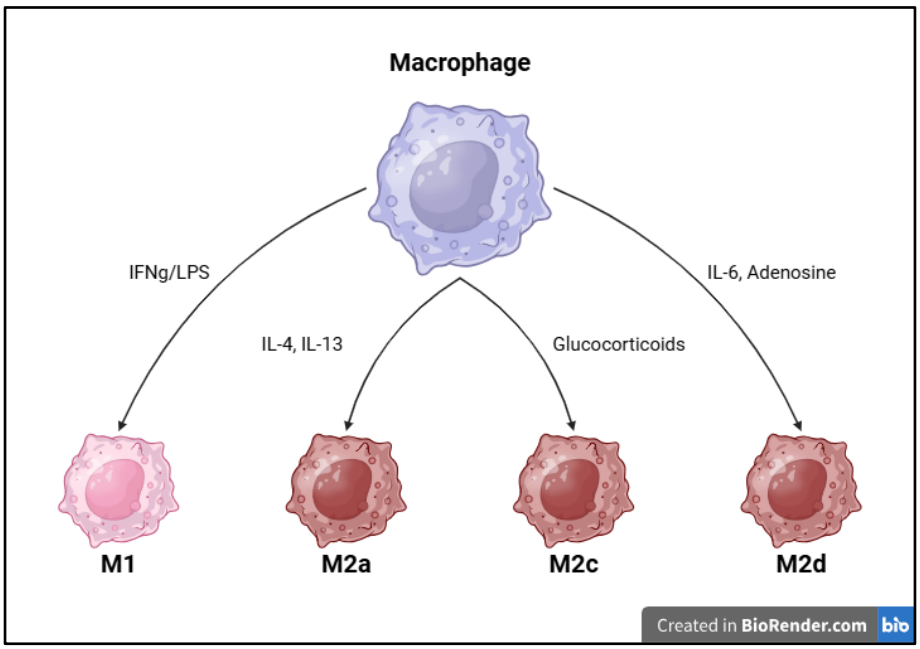

Figure 2. Image of the differentiation process between M1 and M2 macrophages and the respective cytokines necessary to induce each state created using BioRender.com.

Tumour-Associated Macrophages (TAMs) and Multiple Myeloma:

Tumour-associated macrophages (TAMs) are a specific type of macrophage that represents one of the main tumour-infiltrating immune cell types. TAMs are divided into two functionally contrasting subtypes: M1, a classically activated antitumour activity that can directly mediate cytotoxicity and antibody-dependent cell-mediated cytotoxicity (ADCC) to eliminate tumour cells; and M2, an alternatively activated macrophage that promotes the occurrence and metastasis of tumour cells, inhibits T cell-mediated anti-tumour immune responses, and promotes tumour angiogenesis, thus leading to tumour progression. Ultimately, they are involved in the promotion of MM cell proliferation and angiogenesis in the bone marrow, often aiding in its growth while also increasing its immunity to various therapies (Pan et al., 2020).

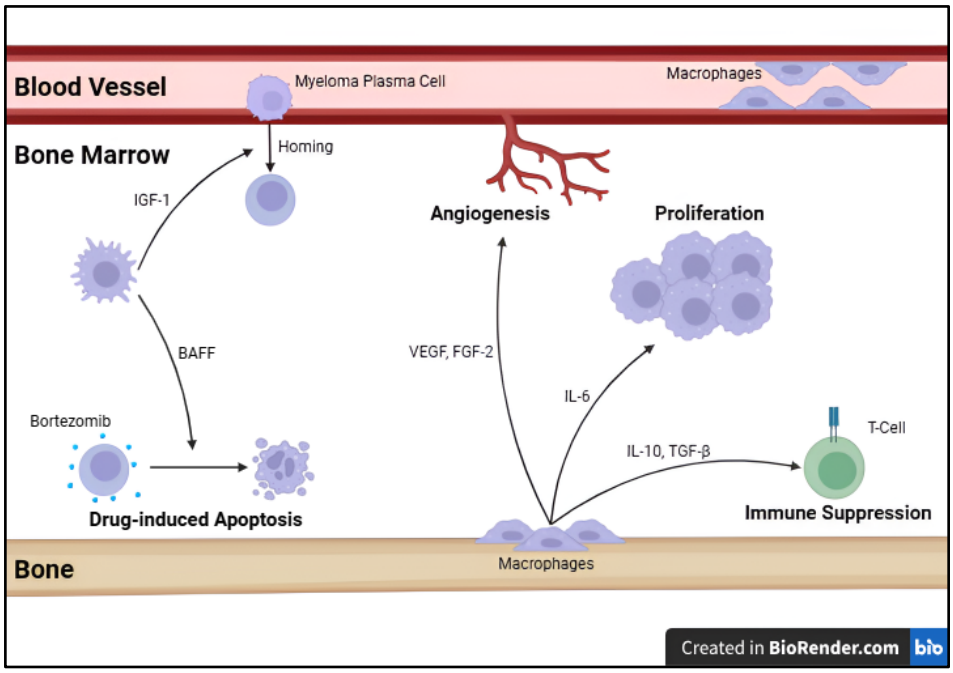

Figure 3. Image of the various functional roles of macrophages in the blood vessel, bone marrow, and bone tissue, created using BioRender.com.

Single-cell transcriptomics and spatial profiling have shown that TAMs are comprised of multiple molecular subtypes within the tumour microenvironment. This is dependent on their spatial localization, tumour type and disease stage, with the six major macrophage clusters being inflammatory macrophages, angiogenic macrophages, immunoregulatory macrophages, interferon-mediated regulator macrophages, immunostimulatory macrophages, and a CD169+ macrophage cluster, whose distinct function has yet to be discovered. Moreover, macrophages are largely associated with a poor prognosis. Historically, their therapeutic targeting has primarily focused on inhibiting their recruitment or reprogramming their phenotype from pro-tumour (M2) to anti-tumour-like (M1), though no ground-breaking clinical breakthroughs have yet to be uncovered. However, several putative therapies are currently actively being tested preclinically to target these functional classes of macrophages, and they may begin to show clinical impact. These therapies largely aim to block the immunosuppressive, inflammatory, or angiogenic activities of these various macrophages. In addition, several emerging studies utilizing single-cell RNA-sequencing (scRNA-seq) and spatial transcriptomics have improved our understanding of the ontogeny, phenotype, and functional plasticity of macrophages. Overall, the recent developments are beginning to show positive signs of expanding our understanding of TAMs, though continued research is necessary (Nasir et al., 2023).

Many studies have illustrated a correlation between TAM and the growth of MM, specifically in its progression and drug resistance. Research has also shown that MM plasma cells in vitro could favour M2 TAM polarization. Additionally, there may exist a possible correlation between the pro-tumour effect of M2 TAMs,

which has been shown to result in reduced resistance to proteasome inhibitors and immunomodulatory drugs. Several clinical studies have proved that CD68/CD163 double-positive M2 TAM is associated with increased microvessel density, chemoresistance, and reduced survival, but notably, this has been determined to be independent of the stage of MM (Cencini et al., 2023).

In a normal environment, macrophages actively participate in maintaining tissue balance. Constantly surveying their surroundings, they patrol tissues to identify and eliminate foreign particles, dead cells, and debris. Specifically, through phagocytosis, they engulf and digest cellular components, preventing the accumulation of waste and contributing to tissue cleanliness. In response to tissue damage, macrophages release growth factors that stimulate cell proliferation and tissue repair, and they collaborate with other cells, such as fibroblasts, in orchestrating the healing process after injury (Gordon & Plüddemann, 2017).

In a tumour environment, tumours release signalling molecules known as chemokines to attract macrophages, leading to increased infiltration. As a result of the tumour microenvironment, changes in macrophage polarization may occur, such as shifts towards a pro-tumour phenotype (M2-like). Additionally, hypoxic conditions produced by tumours may lead to the development of unique macrophage subsets, influencing their behaviour, and macrophages in the tumour microenvironment may adopt immunosuppressive characteristics, inhibiting the anti-tumour response of other immune cells (Gordon & Plüddemann, 2017).

Therapies Targeting TAMs for Multiple Myeloma:

In a 2015 study conducted by Jensen et al., the agonistic anti-CD40 antibody was explored as a potential treatment. Results suggest that CD40, a cell surface costimulatory protein expressed via antigen-presenting cells (APC), is another promising target and is necessary for their activation. Using agonistic antibodies, cancer patients exhibited innate and adaptive immune responses. Specifically, researchers illustrated that the potent anti-MM activity of macrophage-activating immunotherapy was effective in vivo and resulted in the expansion of M1-polarized macrophages within the bone marrow. Moreover, they showed that the concurrent inhibition of Tpl2 kinase promoted the production of the antitumor cytokine IL-12 in vitro and in vivo. Ultimately, the results of the study suggested that an anti-CD40 antibody could be a promising approach as an anti-MM therapy (Jensen et al., 2015).

In a 2018 study conducted by Wang et al., the potential therapeutic effect of the anti-CSF-1T monoclonal antibody was investigated. Results show that in vivo, CSF-1R hindrance could deplete and polarize TAM towards the M1 subtype rather than the M2 subtype, thus inhibiting the growth of MM. Specifically, the results suggested that the CSF1R blockade inhibited the differentiation, proliferation, and survival of murine M2 macrophages and MAMs and repolarized them into M1-like macrophages in vitro. As a result, MM cell growth was inhibited in vivo, which induced a tumour-specific cytotoxic CD4+ T cell response. Ultimately, the results of the study suggest that targeting MAMs with CSF1R-blocking mAbs could be a potentially promising method to promote anti-MM immune responses in patients (Wang et al., 2018).

In a 2019 study conducted by Chen et al., the JAK1/2 inhibitor ruxolitinib demonstrated the ability to suppress M2 TAM by reducing the expression of tribbles homology 1 protein kinase. Specifically, ruxolitinib decreased the expression of CXCL12, CXCR4, MUC1, and CD44 in MM cells and monocytes co-cultured with MM tumour cells, thus aiding in MM patient treatment. Moreover, ruxolitinib also demonstrated the ability to increase the polarization of TAM towards the M1 subtype in vitro or in MM xenograft models in vivo, thus showcasing the ability to restore sensitivity to lenalidomide. Ultimately, the results of the study suggest that JAK1/2 inhibitors such as ruxolitinib could be a potentially promising treatment for MM patients (Chen et al., 2019).

In a 2019 study conducted by Cucè et al., researchers explored the effect of various nanomolecular concentrations of trabectedin on MM cells. Through the comparison of gene-expression profiling (GEP) meta-

analyses of normal and MM plasma cells, they observed an increase in DNA nucleotide excision repair (NER) genes in poor prognosis MM. Specifically, trabectedin induced DNA-damaging response activation, cellular stress via ROS production, and cell cycle arrest. Moreover, results indicated that there was a significant reduction of MCP1 cytokine and VEGF-A in U266- monocyte co-cultures, which confirmed the decrease of an MM-promoting environment, thus downregulating the production of MM cells. Ultimately, the results of the study indicate that the NER-targeting agent trabectedin appears to be a promising candidate as an anti-MM therapy (Cucè et al., 2019).

In a 2022 study conducted by Beider et al., researchers investigated the anti-CXCL13 antibody and its relation with MM proliferation. The results of the study suggest that CXCL13 was one of the most significant increased factors in conjunction with MM growth and that MM cells were the main source of the increased murine CXCL13 levels. By investigating mice inoculated with CXCL13-silenced MM cells, they discovered that the cells developed significantly lower bone marrow diseases and that higher levels of CXCL13 were present in the blood and bone marrow samples of MM patients in contrast with healthy individuals. Ultimately, the results of the study suggest that the suppression of CXCL13 within MM patients would reduce the proliferation and severity of MM cell growth, thus allowing it to potentially serve as a novel MM therapy (Beider et al., 2022).

Conclusion:

In conclusion, though macrophages play a significant role in maintaining a stable state within the human body, there also exists a significant correlation between TAMs and the progression of MM within patients. Several studies indicate that TAMs play a critical role in fostering the tumour environment necessary for MM proliferation and survival, thus increasing the severity of the disease. Although scientists have yet to unravel the cause of MM, the preliminary findings of several recent studies have shown that many potential therapeutic strategies targeting the disruption of TAMs are indeed promising and can potentially mitigate the MM microenvironment and thus the proliferation of MM cells. Therefore, future research should continue to focus on exploring the precise mechanisms of TAM-MM interactions and developing targeted therapies that can effectively modulate this dynamic. Ultimately, such advancements hold promise for innovative treatments that could significantly alter the landscape of MM management and improve prognostic outcomes for patients.

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