Potential Multiple Myeloma Therapeutic Strategies through Targeting Macrophages and Mesenchymal Stromal Cells

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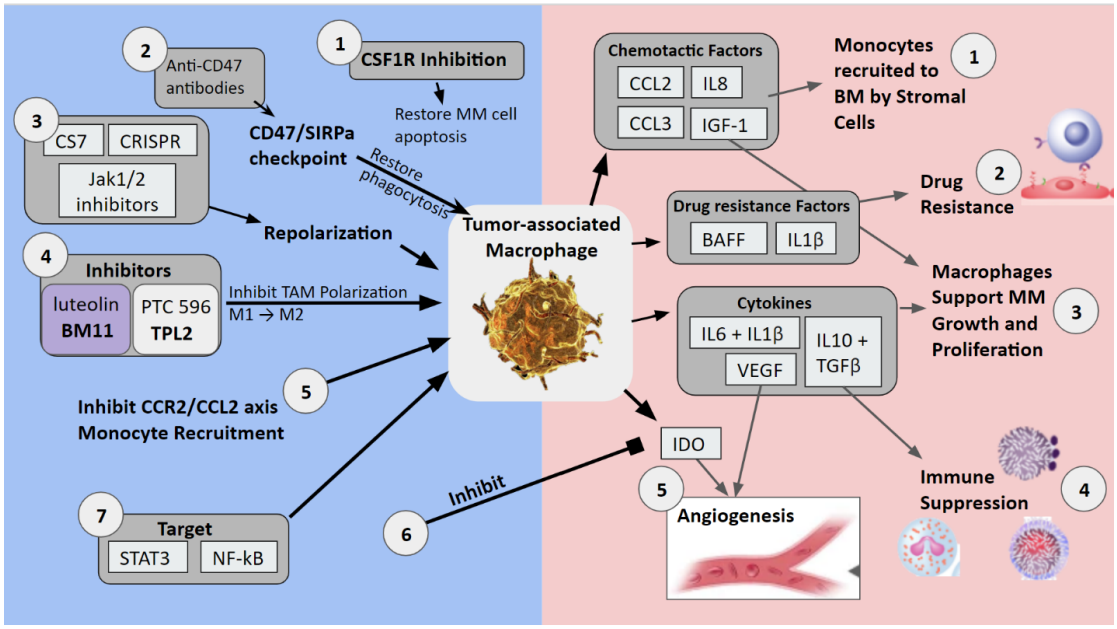
ABSTRACT

Multiple Myeloma (MM), a bone marrow plasma cell hematopoietic cancer, remains a critical but incurable hematological malignancy, prone to deadly relapses even after existing treatment. In this review, I describe the origins of pro-tumor myeloma-associated macrophages. I specifically outline how classically activated anti-tumor macrophages that home to the cancerous bone marrow tumor microenvironment is polarized into pro-tumor macrophages. We then explain how these myeloma-associated macrophages play an important role in supporting multiple myeloma by enabling drug resistance, improved growth, angiogenesis, and protection. We also describe several treatments in development aimed to sever the supportive link between myeloma-associated macrophages and MM by blocking signaling pathways, destroying, or repolarizing macrophages and even preventing macrophage polarization in the first place. We conclude that careful study is needed to improve the reliability of targeting myeloma-associated macrophages to reduce MM relapse and comprehensively treat this cancer.

Visual Abstract



Introduction

Multiple myeloma (MM) is the 2nd most common hematological neoplasm in the US and makes up 13.6% of worldwide hematological cancer cases [1, 2]. It is an incurable hematopoietic plasma cell malignancy that causes 34,470 adult diagnoses per year in the US and 168,765 diagnoses per year in the world [3, 2]. Existing treatments (e.g. immunotherapy and chemotherapy, thalidomide analogs, proteasome inhibitors) only delay mortality; 40% of MM patients do not survive 6 years [3]. Ultimately patients suffer from renal insufficiency, bone lesions, hypercalcemia, and anemia, leading to relapses (median time to relapse after first-line treatment is only 26.9 months) and mortality [4].

One major reason for this unsatisfactory situation is that existing treatments do not adequately account for myeloma-associated macrophage and bone marrow (BM) mesenchymal stromal cells' (MSCs) contributions to myeloma cell survival. Macrophages and MSCs are essential components of the tumor microenvironment and important tumor progression mediators. Over the last decade, scientists have more clearly defined the ways macrophages and MSCs worsen cancer. However, further research is needed to achieve a deeper understanding of the exact methods of macrophage-MM support. This understanding can help scientists discover ways to break this link and improve MM patients' survival chances.

In this review paper, I summarize five (**5**) ways macrophages develop in the tumor micro environment and support myeloma cell growth including: 1) monocyte recruitment by BM stromal cells, 2) improving MM anti-apoptosis ability via drug resistance, 3) supporting tumor cell growth/proliferation, 4) contributing to immune suppression by secreting immune-inhibitory cytokines, 5) bringing nutrients and oxygen to the tumor via angiogenesis and waste removal

I then explain seven (**7**) potential solutions to halt macrophage support for myeloma: 1) blocking Colony Stimulating Factor 1 Receptor (CSF1R), 2) restoring phagocytosis in myeloma-associated macrophages, 3) repolarizing myeloma-associated macrophages back to M1 state, 4) inhibiting macrophage polarization towards myeloma-associated state, 5) inhibiting monocyte recruitment via the C-Chemokine Motif Chemokine Ligand 2 (CCL2) pathway, 6) inhibiting indoleamine 2,3-dioxygenase (IDO) and 7) targeting the STAT3 and NF-kB pathways.

Origins of Myeloma-associated Macrophages

Monocytes normally polarize into 2 types of macrophages: M1 and M2. M1 macrophages (polarized by LPS and IFN-y) are at the forefront of fighting pathogens [5]. M1 cells release IL1b, IL6, IL8, IL12 and TNFa to promote inflammation, they secrete nitric oxide synthase and reactive oxygen species to destroy cancerous cells and they present antigens to activate anti-tumor immune response [6].

By contrast, M2 macrophages (polarized by IL4) exert healing and immunosuppressive functions. M2 cells release VEGF and TGF-b to activate fibroblasts and contribute to wound healing [7, 8]. When macrophages are polarized into M2 after the infection is controlled, they can be helpful in reducing inflammation and bringing surrounding tissue back to normality. When polarized into M2 within the tumor microenvironment, however, M2 macrophages inadvertently strengthen tumor cell survival, proliferation, and growth, ultimately contributing to tumorigenesis.

MM cells hijack M2 macrophage function to strengthen MM growth. Myeloma cells recruit circulating monocytes to the myeloma microenvironment via monocyte attractant chemokines (CCL2, CSF-1, VEGF, FGF2). Inflammatory cells add to chemokine production by secreting FGF2, VEGF and HGF [9]. Once monocytes are recruited by these chemokines, they are then polarized into myeloma-associated macrophages, formally defined as all macrophages within the bone marrow microenvironment. Existing macrophages are also polarized from M1 into myeloma associated macrophage state. Myeloma associated macrophages function similarly to M2 macrophages, supporting growth rather than destroying cancerous cells (biomarkers: CD68, CD163, CD206, arginase expression). They can be recognized due to secretion of IL6, IL10, IL12, TNF-alpha, VEGFa and VEGFc [10].

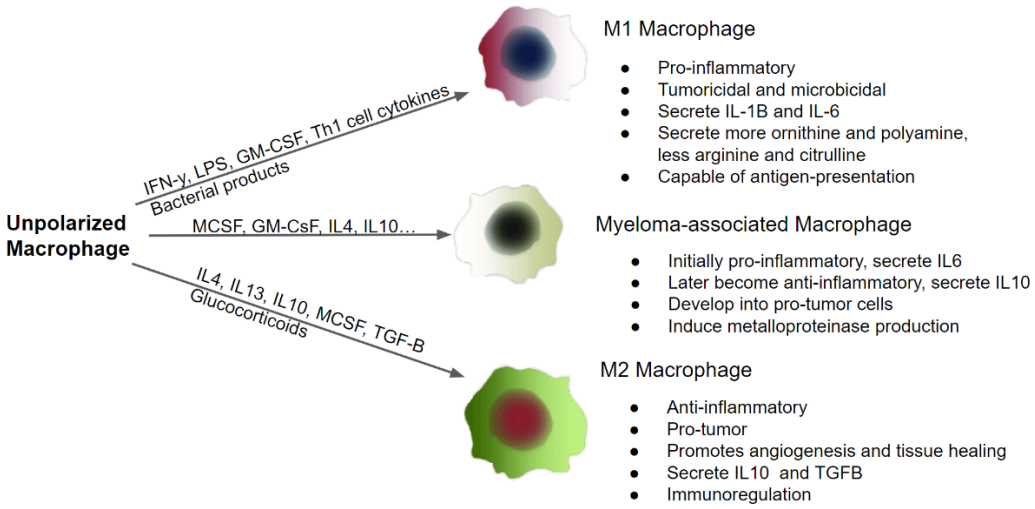


Figure 1. Macrophage Polarization Into M1, M2 and Myeloma-associated Macrophage states. Once monocytes develop into M0 stage macrophages, they can be polarized into M1 or M2 by different stimulation factors. M1 macrophages are pro-inflammatory, microbicidal, tumoricidal, can present antigens and induce a T_H1 response though they also cause tissue damage in the process. M2 macrophages on the other hand are anti-inflammatory, help clear dead tissue and parasites, induce a T_H2 response, regulate and restrict the immune system and induce angiogenesis and tumor promotion [11]. Myeloma-associated macrophages start out like M1 macrophages during the early stage of MM development. As tumorigenesis progresses, the myeloma-associated macrophages move towards M2 characteristics.

IL4, IL13, IL10 and glucocorticoid signaling in the cancerous bone marrow causes the percentage and total number of myeloma associated macrophages to increase compared to M1 macrophages [12]. This is indicated by increased presence of M2 macrophage markers CD206 and CD163 [13].

How Myeloma-associated Macrophages Support MM Tumorigenesis

The tumor microenvironment is essential for multiple myeloma (MM) growth by providing survival signals while secretion of growth and proangiogenic factors. This function is enhanced by the niche support of myeloma-associated macrophages, defined as all macrophages in the bone marrow tumor microenvironment. First, peripheral blood monocytes are recruited to the bone marrow. This recruitment is enhanced by multiple myeloma interacting with Bone Marrow Stromal Cells (BMSCs). Next, the macrophages are polarized and activated by MM cells and mesenchymal stromal cells. Then, the myeloma-associated macrophages secrete growth factors like IGF1, CCL2, CCL3, IL8, proteolytic enzymes, cytokines and inflammatory mediators to support MM cell migration, proliferation and survival [5]. This function amplifies as the tumor progression continues.

Clodronate liposome studies provide experimental evidence that myeloma-associated macrophages correlate with worse MM outcomes. Clodronate liposomes are double membranes containing a 5 mg/mL concentration of the drug clodronic acid (see Figure 2) [14]. When they are ingested to macrophages (intravenously), these liposomes release the lethal clodronate drug, killing macrophages. Specifically, clodronate liposomes target mature CD11b+, F4/80+ CD169+ myeloma-associated macrophages. They also exert a downstream effect on the BM microenvironment composition. Furthermore, clodronate liposomes can impair multiple myeloma cell migration via reducing bone marrow IGF-1 and CXCL12 [15].

For macrophages, clodronate liposome mediated treatment can cause body-wide macrophage depletion which leads to a >95% lower tumor burden after 4 weeks [15]. Clodronate liposomes mediated macrophage depletion

led to decreased 5TGM1 MM cell homing to bone marrow (2.7 fold reduction in total tumor cell number in the BM). Studies also found an increase in tumor cell retention in circulation 24 hours after intravenous tumor inoculation [15].

This works because clodronate liposomes will deplete myeloma-associated macrophage count. Myeloma-associated macrophages secrete IGF-1, CCL2, CCL3 and IL8 chemotactic factors that cause 5TGM1 myeloma cell migration out of circulation to the bone marrow [5]. Therefore, reduced myeloma-associated macrophage count will decrease 5TGM1 myeloma cell numbers in the BM.

Clodronate liposome treatments have already been shown to decrease tumor expansion in other cancers (melanoma, lymphoma, lung adenocarcinoma, ovarian) [15]. This strengthens the case for targeting multiple myeloma to treat multiple myeloma cancer patients.

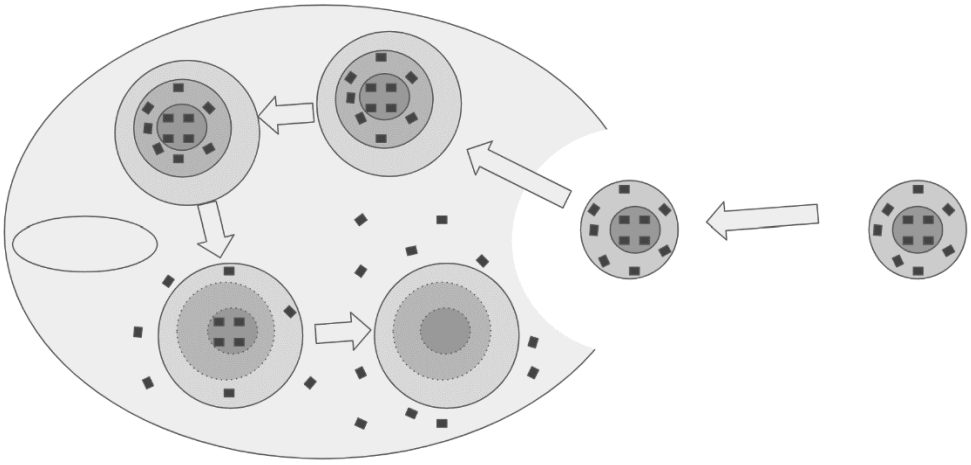


Figure 2. Clodronate Liposomes Function Leads to Macrophage Apoptosis After Ingestion. Double membraned liposomes containing the clodronate chemical lethal in high concentrations to macrophages are ingested by macrophages. The membrane lipids are digested by lysosome phospholipases, releasing clodronate. Once clodronate builds up to a certain concentration, it causes macrophage apoptosis.

Myeloma-associated macrophages are critical to enabling MM survival through five main pathways: BM stromal cell mediated to recruit monocytes into the tumor microenvironment, anti-apoptosis drug resistance, MM cell growth, angiogenesis, and immune suppression.

BM Stromal Cell Mediated Monocyte Recruitment

BM Macrophages and stromal cells produce IGF-1, CCL2, CCL3 and IL8 chemotactic factors that induce MM cells to migrate to the BM and adhere to endothelial cells [5].

A 2015 Cleveland Clinic study finds that both malignant and especially nonmalignant plasma cells overexpress three chemokines CCL3, CCL14 and CCL2. This is significant because these three chemokines' expressions are directly correlated with the presence of full myeloma. An ELISA measurement of chemokine concentration in the bone marrow plasma underlined the importance of chemokines to developing into full myeloma. Whereas control and MGUS study groups demonstrated 5ng/mL of CCL3, 8ng/mL of CCL14 and 60 ng/mL of CCL2, the myeloma experimental group showed major increases in all three chemokines: 15 ng/mL of CCL3, 35 ng/mL of CCL14 and 90 ng/mL of CCL2. This reinforces the idea that myeloma cells induce monocyte migration to the bone marrow via these chemokines. One mechanism for inducing migration could be the activation of PI3K, Akt, Erk and MAPK pathways and c-myc expression due to overexpression of the above three chemokines [16].

The mechanism for inducing monocyte migration also relies on increasing production of the chemokines. CCL2 (monocyte attractant protein), CXCL2, CCL18, CCL9 and CSF1 to recruit more monocytes. Next, factors like CSF-1, GM-CSF and Flt-3 induce the recruited monocytes to differentiate into M2-like phase [16].

Additionally, CXCL12 (CXC Chemokine Ligand 12) mediates monocyte recruitment into the tumor by binding to CXCR4 (CXC Chemokine Receptor 4) present on monocyte membranes. Evidence for the presence of CXCR4 on monocyte/macrophage membranes is supported by the increased number of CXCR4 expressing macrophages detected in MM patient bone marrow compared to MGUS and normal patients. After recruitment, MM cells then promoted M2 receptor CD206 and blocked LPS-induced TNF-a secretion, weakening an antitumor M1 response. These actions induced recruited monocytes to polarize into M2 style myeloma-associated macrophages rather than M1 macrophages [17].

Drug Resistance

Standard MM treatment drugs like melphalan, bortezomib, dexamethasone and lenalidomide directly destroy MM cells via inducing apoptosis [11]. Pro-tumor macrophages (especially M2 and myeloma-associated) protect against this effect through several methods.

Pro-tumor macrophages emit B cell activating factor (BAFF) cell survival factor via direct contact through the P-selectin ligand.[14] BAFF inhibits apoptotic signaler function via NF-kB pathways to prevent MM cells from undergoing apoptosis. Furthermore, myeloma associated macrophages secrete IL-1B, which promotes increased MM stem cell differentiation as 2017 Zhejiang University research shows. Both IL-1B and BAFF, especially negate bortezomib's effects [18]. Cell contact-mediated mechanisms like P-selectin ligand and ICAM-1/CD18 induce resistance to melphalan, as demonstrated in an MD Anderson Cancer Center study [19].

2015 Vrije Universiteit Brussels research demonstrates how myeloma-associated macrophages protect specifically against the anti-cancer drugs melphalan and bortezomib. The study's target was STAT3: a crucial link between cancer and inflammation as one of the most activated oncogenic transcription factors in both cancer cells and micro-environmental myeloid immune cells [13].

Myeloma-associated macrophages induce STAT3 activation leading to less caspace-3 cleavage, resulting in decreased cancer cell apoptosis. Thus, STAT3 activation provides another channel through which myeloma-associated macrophages protect myeloma cells [13].

A 2017 Zhejiang clinical study demonstrates the practical results: high myeloma-associated macrophage frequencies in the BM negatively correlate with survival outcome: even as the patient is given dexamethasone chemotherapy [18]

Macrophages support MM Growth and Proliferation

Myeloma-associated Macrophages release IL6, IL10, IL 1 beta, VEGF, TGF-b and IGF-1 [5]. These cytokines upregulate the JAK/STAT3 signaling pathway, which ultimately induces MM cell proliferation and promotes MM cell growth and survival [20]. Some of these cytokines also upregulate production of other related cytokines (e.g. IL6 leads to more IL10 production), amplifying the effect.

This correlation between myeloma associated macrophage presence and MM cell development has been proven through several studies. A National and Kapodistrian University of Athens study shows that the presence of myeloma-associated macrophages is one of the markers most correlated with patients failing to achieve cancer remission [21]. Furthermore, A 2014 Aarhus University experiment shows that the presence of soluble CD163 (a myeloma associated macrophage marker) in a patient's serum is associated with poor survival rates) [22].

Angiogenesis

Macrophages produce IL10, FGF2, MMPs, CCLs and most significantly: VEGF, which cause themselves (macrophages) to express genes to produce Tek, FVIII-RA, VEGFR2, VE cadherin, Tie2 and FGFR2. Expression of these

genes allows macrophages (e.g. Tie2+ types) to mimic vascular tissue (forming capillary-like neo vessels) and bring nutrients to the tumor [23]. Other macrophage released molecules like iNOS (increases blood flow to tumor) and TNF alpha (remodels blood vessels) improve angiogenesis. This means the MM cancer tumor receives more nutrients and oxygen via blood, increasing its growth and diverting resources away from normally functioning cells [24].
Several studies demonstrate myeloma associated macrophages' roles in angiogenesis:

A 2015 San Raffaele Scientific Institute study demonstrated that specific macrophage types, like CD206+ and Tie2+ expressing macrophages are correlated with an increase in micro vessel density and angiogenesis-supporting cytokines [25]. Likewise, a Hospital of Heraklion experiment in the same year showed that IL10 produced by myeloma associated macrophages is correlated with more angiogenic cytokine presence [26]. An in vitro study has also shown that both myeloma cells themselves and associated macrophages can promote human umbilical vein endothelial cell growth [27]. This lends credence to the idea that in vivo myeloma associated macrophages can promote vascular endothelial cells growth to bring blood vessels into the tumor.

Immune Suppression

When macrophages arrive in the BM tumor microenvironment, they (macrophages) lose the ability to present antigens, phagocytize and stimulate the adaptive immune response.

How exactly does immunosuppression occur? Macrophages in the tumor microenvironment express Indoleamine 2,3-dioxygenase (IDO) and Interleukin 10 (IL10). IDO inhibits effector T cell function and promotes differentiation of T regulatory cells (which suppress phagocytosis) by degrading the tryptophan amino acid. IL10 in turn inhibits expression of MHC class 2, a lymphocyte surface protein critical to presenting antigens to CD4 T cells for stimulating anti-tumor adaptive immunity. IL10 also increases cytotoxic T cell factor (e.g. granzyme B, IFN-y, eomesodermin) expression [28].

Both IDO and IL10 function leads to less effector T cell activation and a weaker adaptive immune response against cancer, benefiting the tumor. In addition, MM cells themselves express "do not eat me signals" (CD47) which inhibits phagocytosis by macrophages [29].

Mesenchymal Stromal Cell Role

Mesenchymal Stromal Cells (MSCs) provide homing signaling CXCL12 attracting MM migration to bone marrow and provide contact-mediated support for MM via cytokines like IL-6, VEGF-a, IGF-1, CCL5, Interferon y and help MM development via cell adhesion. MSCs also polarize macrophages into MSC-activated macrophages (express more IL10, IL6, less IL12, TNF-a). The newly polarized myeloma associated macrophages display characteristics between M1 and M2 macrophages and promote angiogenesis and reduce LPS responsiveness [30].

Myeloma-associated macrophages in return provide enhanced motility for MSCs and induce them to produce IL6, CCL5 and Interferon-y. Myeloma cells also use TNF-alpha-mediated CCL2 induction to upregulate BM MSC function [30].

Potential Therapeutic Strategies

How can medicine prevent myeloma-associated macrophages from supporting MM and reduce minimal residual disease and relapse? There are 7 potential solutions:

CSF1/CSF1R Blockade to Inhibit Myeloma-associated Macrophage Development

Targeting or blocking the CSF1R (colony stimulating factor 1 receptor, see Figure 3) can decrease CD68+, CD103+ levels and restore macrophage apoptosis [31]. Colony Stimulating Factor 1 (CSF1) is a ligand released from cancer cells that stimulates ERK (Extracellular receptor kinase) 1/2 phosphorylation in macrophages. This CSF1-induced phosphorylation plays a role in polarizing macrophages from anti-tumor M1 state to the harmful M2 myeloma-associated pro-tumor state.

Two main methods of blocking CSF1R are 1) monoclonal anti-CSF1R like emactuzumab, CS7, IMC-CS4 and 2) CSF1R pharmacological inhibitors like ARRY382, JNJ-40346527, BLZ945 [32]. An example of the practical potential of CSF1R blockade drugs is FDA-approved PLX3397 (pexidartinib) used to treat sarcoma. Pexidartinib suppresses ERK stimulation by CSF1, preventing macrophage polarization into myeloma-associated state. This practical application in sarcoma indicates drugs using a similar CSF1/CSF1R blockade mechanism could be used in multiple myeloma [33].

A 2018 in vitro study at Jilin University First Hospital shows that this treatment inhibits differentiation, polarization, and survival of M2 and myeloma-associated macrophages, sometimes repolarizing myeloma-associated macrophages back to M1 state. The physical results of CSF1R blockade are demonstrated by an in vivo mouse study which showed that blocking CSF1R decreases myeloma growth in vivo by blocking monoclonal antibodies [33].

There are two things to note about CSF1R inhibition. Firstly, CSF1R inhibition has few toxic side effects, reducing patient harm. Secondly, CSF1R inhibitors work best when they are combined with drugs like bortezomib or melphalan, meaning they won't interfere with other treatment's function [33].

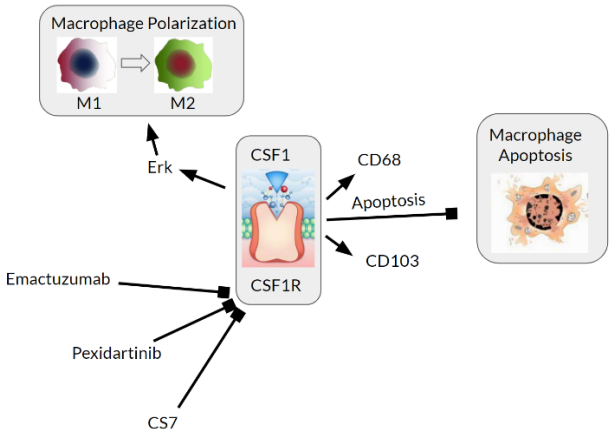


Figure 3. Colony Stimulating Factor 1 Receptor Inhibition Mechanism. Colony Stimulating Factor 1 (CSF1) binding to its associated receptor (CSF1R) on macrophages is a critical mediator promoting macrophage polarization towards myeloma-associated M2-like phase, expressing CD68+ and CD103+ state and inducing macrophage apoptosis. Several monoclonal anti-CSF1R antibodies and drugs target CSF1R for this reason.

Block CD47/SIRP1 Checkpoint to Restore Macrophage Phagocytosis

During normal function, immune checkpoints serve as necessary, measured restrictions on immune cell function. MM, however, misuses these checkpoints as inhibitory mechanisms to prevent the immune system from recognizing and destroying cancer. Common T cell immune checkpoint therapies targeting the PD-1/PD-L1 and CTLA-4 checkpoints have not shown much efficacy against MM [34]. Instead, targeting immune cells in macrophages or the CD47/SIRPa checkpoint has potential as a treatment. Anti-CD47 antibodies (like Hu5F9-G4) can restore macrophages' ability to phagocytose MM cells like normal. These antibodies will inhibit "don't eat me" signals and block the CD47 immune

checkpoint protein [35]. This method has led to reductions in other cancers like acute myeloid leukemia, non-Hodgkin lymphoma, pancreatic cancer and small lung cancer, indicating potential viability against MM.

What is the current state of immune checkpoint targeting drug development? Hu5F9-G4 has the most potential out of existing treatment options - it is entering phase 2 clinical trials. Other molecules under investigation include the SIRPa-IgG1 Fc fusion protein TTI-621 have also shown antitumor effects in an MM xenograft [36]. TTI-622 has demonstrated efficacy in combating advanced relapsed myeloma and is noted for inducing a durable response and working best along with carfilzomib and dexamethasone drugs. However, existing data is from lymphoma patients - further studies with myeloma patients are needed to confirm function. Other anti-CD47 monoclonal antibodies like AO-176 (in Phase 1/2 trials) and SRF231 (in Phase 1a/1b trials) are under development as well [37]

Not only monoclonal antibodies, but also microRNA treatments are under consideration. One miRNA candidate is miR-155, which directly regulates CD47. miR-155 can be artificially overexpressed, which suppresses CD47 expression and restores myeloma-associated macrophage phagocytosis function [38].

TAM Repolarization Towards Anti-Tumor M1 macrophages

Repolarizing TAM macrophages toward M1 macrophages via low doses of anti-CSF1R antibody CS7, Jak1/2 inhibitors (like AZD1480 or ruxolitinib) or CRISPR/Cas9 reprogramming along with CD40 priming and TLR triggering can reduce M2 macrophage numbers [39, 40] This reduction is accompanied by an increase in M1 macrophages, leading to a beneficial CD4+ T cell immune response.

For instance, a 2016 Madrid University School of Medicine study developed a double treatment strategy [41]. Researchers simultaneously added pro-M1 cytokine granulocyte-macrophage CSF and blocked pro-M2 factors in a human MM cell line mouse xenograft model. Likewise, an Institute for Myeloma & Bone Cancer Research study demonstrated how the Ruxolitinib inhibitor molecule in vitro and in vivo reduces Tribbles homolog 1 protein kinase expression to repolarize M2 macrophages back to M1 state [39]. Finally, the efficacy of CD40 was demonstrated in a 2015 University of Wisconsin-Madison preclinical study which found CD40 activation followed by TLR ligation successfully provoked an innate immune response against MM [39]. This works by targeting CD40 (antigen presenting cell surface costimulatory protein) via agonistic antibodies. Strategies like these can help restore macrophages back to their original anti-tumor expected function.

Repolarizing myeloma-associated macrophages is a promising direction. However, existing research on this method has been *in vitro* or using *in vivo* xenografts. Additional in vitro studies are needed to determine viability.

Inhibit Polarization from M1 to M2 State

In addition to restoring macrophages to M1 state, inhibiting their polarization away from M1 state in the first place is a potential option. One method to achieve this is to halt signals that shift macrophages to M2, keeping them as M1 macrophages.

The Tumor Progression Locus 2 (TPL2) kinase protein (Figure 4) makes an especially ideal target due to its unique structure in its ATP binding loop compared to other proteins which allows researchers to design specific proteins to target the kinase [42]. TPL2 blocking molecules like luteolin have been in development for over a decade but further research to accurately determine the TPL2 crystal structure is needed.

BM11 secreted by myeloma cells to influence macrophage development is also a potential target. BM11 promotes angiogenesis, chemoresistance and growth. A 2021 Zhang et al. experiment shows that inhibitors like PTC596 can prolong mouse survival by reducing BM11 induced macrophage function [43].

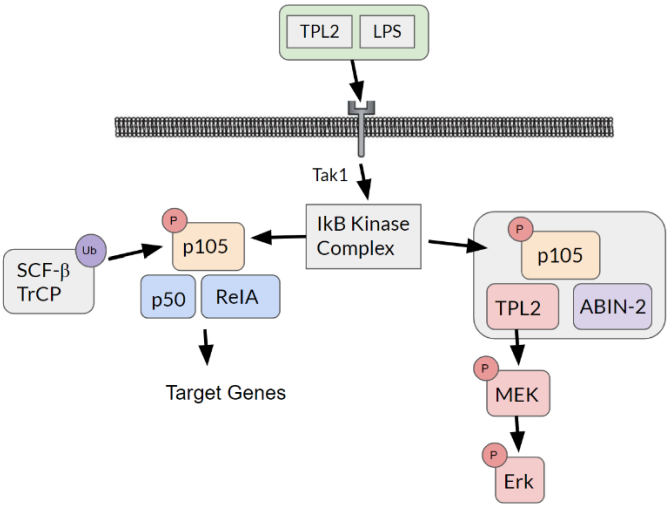


Figure 4. Tumor Progression Locus 2 (TPL2) Kinase Phosphorylation Mechanism. The TPL2 kinase is linked with the NF-kB inhibitor (NF-kB p105). When NF-kB p105 is phosphorylated, it induces in turn phosphorylation of MEK and finally Erk to induce macrophage polarization towards M2-like myeloma-associated macrophage phase. This makes TPL2 a key target for blocking molecules.

Inhibit Monocyte Recruitment

Interfering with monocyte recruitment to the MM niche via CCL2-CCR2 axis inhibition [44]. Both CCR2 inhibitors and anti-CCL2 antibodies can achieve this function.

CCR2 (C-Chemokine Receptor 2) is the receptor for CCL2 (C-Chemokine Motif Chemokine Ligand 2), a ligand released by monocytes to trigger their recruitment to the tumor (driving homing and chemotaxis) and common in the myeloma BM vascular network. Inhibiting CCR2 with monoclonal antibodies can reduce macrophage movement to the tumor. Targeting the CCL2/CCR2 axis in a 2017 Chinese Academy of Sciences study has proven results in other tumor types such as liver, pancreatic and bladder cancers [44]. Research on CCR2/CCL2 inhibition has only recently entered the myeloma cancer field, but if proven, it could help MM recovery.

The CXCL-12-CXCR4 axis is also crucial to recruiting macrophages to the cancerous BM. MM cells express the CXCL-12 chemokine so inhibiting CXCR4 via a neutralizing antibody can reduce the number of monocytes recruited and differentiated into M2 type.

Inhibit Macrophage-related Molecule IDO

In addition to targeting myeloma-associated macrophages themselves, researchers can also study how to mitigate the harmful molecules they produce. Indoleamine - pyrrole 2,3-dioxygenase (IDO) is a harmful molecule produced by myeloma-associated macrophages. IDO inhibits lymphocyte T cell proliferation, reducing the adaptive immune response [45]. It also upregulates T regulatory cells to block immunity and decreases the body's cytokine production.

Myeloma-associated macrophages produce IDO via binding to Proteinase 3 on macrophages and activating STAT3 and NF-kB pathways. A first treatment for this is to target Proteinase 3 and inhibit the STAT3/NF-kB pathway. Other options include directly targeting the IDO molecule via D-L-1-methyl-tryptophan. Targeting molecules like IDO can stop the proximate damage caused by myeloma-associated macrophages [45].

Targeting STAT3 to Inhibit Myeloma-associated Macrophages

A 2015 Vrije Universiteit Brussels study using the murine 5T33MM model reported that myeloma-associated macrophages aided myeloma cell survival via STAT3 activation [13]. Specifically, STAT3 activation leads to reduced cleavage of caspase-3, leading to less macrophage apoptosis. Myeloma-associated macrophage-mediated MM cell survival can be abrogated by STAT3 inhibition.

Which target leads to best results when aiming to inhibit STAT3? Researchers tried targeting IL-6 and IL-10, proteins that induce STAT3 activation. However, blocking via anti-IL6 and anti-IL10 antibodies did not result in major decrease in macrophage-induced cell survival [13].

Experiments find the Jak2 inhibitor AZD1480 to be a preferable target. AZD1480 inhibits the Jak2 pathway by competitively interfering with the Jak2 protein's ATP receptor sites. Blocking Jak2 would reduce myeloma's immunity to the standard anti-myeloma drug bortezomib. Thus, a combination of AZD1480 and bortezomib could decrease myeloma drug resistance [13].

Discussion & Conclusion

The frequent relapses that characterize multiple myeloma would not be possible without assistance from the surrounding tumor microenvironment, particularly myeloma-associated macrophages. These cells originate from monocytes and home to the cancerous BM due to the interplay of various chemotactic factors. Once there, they play a major role in supporting myeloma via inducing MM cell homing to the BM, supporting tumor cell growth, increasing drug resistance, bringing nutrients and oxygen to the tumor via angiogenesis and suppressing the immune response. This makes myeloma-associated macrophages a critical target for comprehensive MM treatment.

Rather than solely focusing on tumor eradication, oncologists could move to simultaneously target the myeloma-associated macrophages which aid malignant biological pathways within cancer. In practice, this could mean blocking the CSF1R, CD47 or CCR2 pathways, inhibiting polarization towards myeloma-associated state or repolarizing macrophages back to classically activated state or targeting IDO, TPL2 or STAT3. Treating only cancer cells while leaving its tumor microenvironment support network, including myeloma-associated macrophages, intact risks further relapse. Breaking the link between myeloma-associated macrophages and tumor cells has the potential to provide a more complete treatment toolkit for long term multiple myeloma survival.

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