Multiple Myeloma : a bone eating disease

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Abstract

Multiple myeloma (MM) is an incurable malignant neoplasm of plasma cells that are terminally differentiated. The disease is characterized by a disrupted immune system, anemia, bone destruction and organ damage. Elderly people are predominantly affected and have an overall 5-year survival rate close to 50%. The malignant plasma cells are located in the bone marrow, where the environment allows for uncontrolled growth. The bone marrow microenvironment (BMM) homes multiple chemical signaling-stromal cells that induce plasma cell proliferation and angiogenesis. Current treatment option are very expensive and often fail to prevent relapse of the cancer. Monoclonal gammopathy of undetermined significance, a pre-malignant state of MM, is asymptomatic and never progresses to MM in a vast majority. Prevention is always preferred over treatment of symptoms, interfering with the progression to MM could therefore be the solution. The heterogeneous mutational landscape of MM tumors shape diverse genetic alterations, but lack consistent mutational hallmarks which cause disease onset. This thesis reviewed how prevention of the progression to multiple myeloma can be achieved by targeting cell-interactions in the bone marrow microenvironment.

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Introduction

One of the first well-documented reports on Multiple Myeloma (MM) was published in 1844. The report described the second worldwide known patient to be diagnosed with MM, by S. Solly. The patient, a 39-year old woman, had growing bone-pain symptoms accompanied with weariness [2]. Four years after the onset of the symptoms, an autopsy was performed on the deceased woman, the autopsy revealed replacement of the bone marrow with a red substance infiltrated by odd-looking cells and destructed bones [3]. The incurable hematological-disease is characterized by an accumulation of malignant plasma cells (MPCs) in the bone marrow, causing anemia, bone-pain, kidney-damage, weariness and organ failure in the end. It is predominantly existent in older people, the median age at diagnosis is 69-years with a 5-year survival rate of 52.2%[4]. MM is responsible for a predicted 2,1% of all cancer-related deaths in the U.S. [4]. Afro-American males have a two-times higher likelihood to develop MM when compared to other races, but it remains unclear why [3]. The incidence rates of MM are believed to increase due to the prolonged aging of the population. There exist several case reports on MM in young people, although 19-40 years old patients account for less than 2% of all MM cases, and while extremely rare, the disease is equally lethal for this younger patient group [5].

It is believed that genetic events and environmental factors are part of the cause and progression of the disease. Based on the lifestyle, multiple cohort studies have examined the effects of alcohol and tobacco, but suggested no causal association with MM [6]. The diet seems to be a risk factor in MM, especially for people that develop diet-induced obesity [7]. Obesity-related molecular factors have been proven to contribute to the carcinogenesis of common solid tumors, which also play a role in MM [8]. The gut-microbiome might also be associated with the progression to MM, as several intestinal microbes cause mucosal-inflammation which drives autoimmunity malignancy [9]. Additionally, a reduced vitamin D level is often found in MM patients, although it remains unclear if it is a disease biomarker or driver [10-11]. However, more precise studies have to be performed in order to discover a possible relation, focused more on the progression to MM and what drives it.

Monoclonal gammopathy of undetermined significance (MGUS) is the asymptomatic pre-malignant stage of MM. It is frequently discovered by accident during clinical follow-ups. When there is a suspicion about a patient having a clonal cell disorder, a screening for monoclonal (M) protein will serve as measurement (*figure* 1) for the tumor mass and staging of the patient [12]. The malignant monoclonal plasma cells (-MPCs) produce monoclonal immunoglobulins (M-spike) which obstinate the renal tubules with light chains causing renal insufficiency. The MPCs tend to accumulate in the bone marrow to embed for further growth. Resulting in anemia and an impaired BMM, leading to lytic bone-lesions and hypercalcemia [13]. Although most MGUS patients rarely progress into developing smoldering multiple myeloma (SMM) or MM, there remains a 1% chance each year for patients to progress to MM [14]. Nevertheless, MM is considered as a frequently observed monoclonal disorder while it is the second most common hematologic malignancy in the U.S.[4-15]. SMM represents the proliferative interstate between MGUS and MM. Patients with asymptomatic SMM have an elevated chance for progressing to a symptomatic disease state. This elevates the 1% chance to a 10% chance for developing MM per year, during the first 5 years after SMM diagnosis [16]. MM patients are staged according to the CRAB-criteria, consisting of hyperCalcemia, Renal insufficiency, Anemia and Bone lesions [17-11]. The CRAB-criteria are applied only on MM patients, MGUS and SMM are asymptomatic and do not exhibit symptoms of the CRAB-criteria. The M-protein in the blood serum serves as a key diagnostic marker for staging the disease and progression [18]. Around 80%-90% of all MM patients will develop a certain degree of bone degradation, which allows radio-imaging techniques to diagnose the severity [19]. Imaging lytic bone-lesions is performed through low dose

whole-body CT, this enables the visualization of pre-existing lesions. MRI and PET scans enable focal lesions detection in MM patients. Focal lesions define bone destruction and tumor growth surrounding the brain tissue [20].

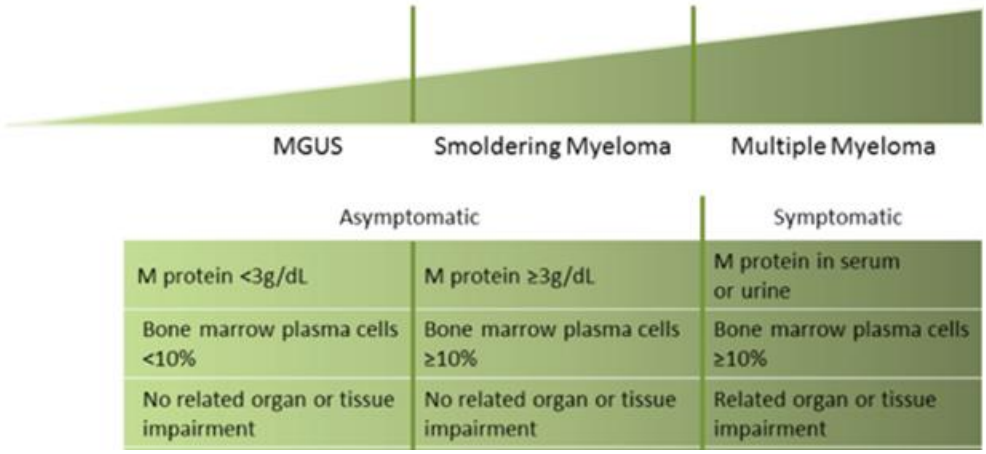

Figure 1: The main criteria that determine the asymptomatic and symptomatic stages of MM [21].

MGUS is associated with the onset of SMM and further progression to MM, but yet, the underlying mechanisms that drive this process are still poorly understood. MGUS already shows genetic alterations that are similar to MM. However, without secondary genetic events, MM does not occur [22]. This finding suggested the involvement of another driver that causes disease progression. The tumor environment . The tumor environment is referred to as the bone marrow microenvironment (BMM) in MM. The BMM provides an optimal spot for MPCs to grow and proliferate [23]. Myeloma cells exhibit a heterogeneous mutational landscape that is affected by signaling that came from the BMM [24]. The mutations dysregulate the tumor-suppressive gene expression and enhance oncogenic gene expression. In MM, the variability in genetic changes is very broad [25]. The inconsistent mutational load creates complexity which disables the applicability of a consistent hallmark mutation [26]. The mutational load in the MPCs provides invisibility from immunosurveillance by altering the expression of cell-surface proteins and cytokine secretion. Additionally, the loss of genomic stability that drives the chromosomal disfigurement can also be recognized as a hallmark of MM [27]. The symptomatic stage of the disease is due to bone marrow infiltrations, inflammations and impaired signaling that leads to bone lesions. In healthy subjects the building and breakdown of bone is set at an equilibrium, in MM the breakdown has increased significantly resulting in a net bone loss [28].

The immune cells present in the BMM create a niche for the tumor cells by producing several cytokines, chemokines, growth factors and hormones [28]. Macrophages and neutrophils are predominantly responsible for secreting inflammatory agents, which contribute to the malignant cell growth and are toxic to healthy cells [29-28]. The bone marrow stromal cells induce enhanced plasma cell survival through adhesion molecules, resulting in activation of multiple signaling pathways, supporting tumor growth [30]. Furthermore, the MPCs interact with the endothelial cells to induce neo-angiogenesis which is required for disease progression and tumor sustainability [31-32]. In MM, the immune cells that normally clear harmful factors from the body and the regulatory cells that control inflammation, are tricked by the BMM to remain inactive or contribute to the tumor growth. For example, impaired regulatory T cells (-Tregs) enable an ongoing inflammation and disrupt the immune surveillance for the protection of self-tolerance [23]. Dysregulations in self-tolerance of the immune system is believed to play a role in the development of anemia in MM patients [33].

Although several treatments can induce regression, patients still have to battle with relapses due to clonal evolution of the MPCs. This process dismantles the current treatment plans [34]. Frontline therapy for newly diagnosed patients is improving steadily, longer lasting and deeper regression are the results of newly developed drugs. Current treatments usually consists out of proteasome inhibitors (PIs), chemotherapeutic agents, immune-modulating therapies and autologous stem cell transplantation (ASCT) [35]. When patients inevitable encounter relapse throughout the disease, clinicians have to select another suitable treatment afterwards, this usually consists out of a combination of multiple drugs. The optimal combination and dosage of drugs remains an obstacle still, different mutations and stromal cells require different treatment. Personalized-treatment for each individual would be the most beneficial, but this can only be accomplished through intensive patient screening [35].

The treatment design is complex for MM, preventing the progression of the disease will possibly prevent relapse and the transition of MGUS to MM. Moreover, prevention will extensively improve life-quality for the patient. The financial toxicity for the patients would be reduced and the burden relatives have to carry will scale down substantially [11]. The BMM is a key factor in MM, providing a niche for the disease which already forms during MGUS in a majority patients [28]. Targeting the BMM during treatment could provide enhanced survival outcomes and reduced progression in MM. The failure of most present cures are due to the tumor heterogeneity while the treatments target the MPCs, implying for the BMM to serve as a potential target. The mutual interaction between the BMM and the tumor cells demands better understanding for the development of novel therapeutic strategies with preventive abilities [36]. On the other hand, MGUS usually does not always progress to MM. Thus, requiring sensitive evaluation on whether preventive treatment would be beneficial for the patient.

This review describe how the progression to multiple myeloma can be prevented by targeting cell-interactions in the bone marrow microenvironment. Furthermore, MPCs, the BMM, inflammation, the immune system and current/novel therapeutic strategies will be discussed. These elements are believed to be the key players in MM and offer solutions to the possible prevention of MM, which could scale down the disease.

The malignant plasma cell

Monoclonal plasma cell genetics & deficiencies

The plasma cell forms a crucial factor in the line of defense in the adaptive immune system. The repertoire of the B cell offers protection from infections and other diseases. However, the B cell genome remains susceptible for translocations [32]. Mutations in the immunoglobulin (Ig) loci infrequently lead to a malignant transformation in the B cells. Translocated proto-oncogenes drive the initiation of MM and contribute to the progression by acquiring more mutational load over time [37]. After carrying a correct antigen-specific B cell receptor which enables survival, B cells start to proliferate. During the proliferation, two important molecular alterations occur : class switch recombination (CSR) and somatic hypermutation (SHM) [38]. CSR facilitates the change in isotype to produce specific antibodies [39]. The SHM process drives the acquisition of mutations in the V_H regions, resulting in a diminished intraclonal variation and isotype-switched Ig heavy chain genes [37]. The mutational load creates a hyper-diploid or a nonhyperdiploid genome with spread translocations in the karyotype [22]. In some cases this results in a gain of oncogenic function and secretion of the notorious M-protein (dysfunctional IgG/IgM or IgA mostly) accompanied with light-chain secretion [37]. M-protein related disorders usually involve multiple organs. Amyloid light-chain amyloidosis deposits in tissues as amyloid fibrils causing severe organ damage, especially in the kidneys [40].

Frequently observed mutations in MM are cyclin-D1 translocations t(11;14) and cyclin-D3 translocations t(6;14) [25]. In addition, frequently observed mutations in the heterogeneous landscape of MM are alterations in the KRAS, NRAS, TP53, DIS3, FAM46C and BRAF genes, which are drivers of the disease [25]. A collection of various mutations drives the onset and progression of MM. To complicate the disease even further, the mutational pattern deviates in every patient with a different outcome in each. In MM genetics might not serve as a valid forecasting model due to the large variation in the genetic landscape. This troubles the understanding of the disease while lacking a consistent hallmark without constant progression [41].

An additional consideration in the pathogenesis of MM is the regulation of microRNA-dependent gene expression and mRNA splicing. MicroRNAs are believed to promote intron retention that provide novel malignant characteristics for the MM genome [42]. Therapeutic intervention of this process may improve patient outcome by increasing stability in the splicing network. A disturbed epigenetic regulation is frequently observed in cancer and contribute in the progression and onset of the disease [27]. Numerous epigenetic mutations have been classified through sequencing and gene expression profiling studies, including DNA-hypermethylation of cancer-related and B cell specific genes, genome wide hypomethylation and genetic defects, copy number alterations and abnormal expression patterns [43]. These alterations in the epigenetic profile have been linked to MM progression and drug resistance by increasing the plasticity of myeloma cells [44]. Malignant methylation patterns may even contribute to the development of extramedullary soft-tissue myeloma cells [45]. These extramedullary myeloma cells are independent of the BMM and are highly metastatic [45]. This form of MM is often referred to as secondary plasma cell leukemia, the most aggressive type of MM that induces rapid metastasis and a drastically shortened life-expectancy [46]. Clinical intervention of the epigenetic mutations and the shielding of adhesion molecules might prevent the origin of this rare type of MM. Secondary plasma cell leukemia is usually found in patients after relapse that is induced by drug-resistance [26].

The immune system & inflammation

T lymphocyte activity

The immune system plays a debatable role in MM, a defense mechanism becomes a life-threatening factor for the host. The bone marrow site is often populated with a wide range of leukocytes. Lymphocytes are known to contribute to the progression of MM by secreting multiple pro-inflammatory agents. Consequences of MM consist of sharply reduced activity levels of lymphocytes and strong downregulation of cell-surface costimulatory molecules on the majority of leukocytes [23]. Clonal T cell populations in MM are associated with $CD75^+$ and $CD8^+$ T cell clones with reduced turnover rates combined with lower levels of the CD95 apoptotic marker. This impairs recognition of cell-cell contacts and results in less cellular death [47]. Tumor-associated antigens persistently stimulate the accumulation of $CD8^+$ T cells. Although, without enough expression of the cell death-inducing FAS ligand and other co-stimulatory molecules, the apoptotic stimulus will be impaired. Therefore enabling ongoing inflammation and tumor growth while clearance of myeloma cells is compromised [48].

The innate immune system

The innate immune system is thought to be a crucial factor in the suppression of the anti-tumor immune system. High numbers of tumor associated macrophages (TAMs) have been linked to poor prognosis for hematological malignancies [49-50]. TAMs drive the progression of malignancy by reshaping the matrix and inducing tumor-angiogenesis through a positive feedback loop consisting of IL-6, TNF-α and VEGF [51]. The suppression of the anti-tumor response is mediated through cell-cell contact of PSGL-1/selectin and ICAM-1/CD18 between the myeloma cells and the TAMs. This interaction provide immunity against caspase-independent apoptosis and contribute to chemotherapy-resistance [52-50].

A controlling mechanism against the progression is derived through NK cells, NK cells are able to identify down-regulation of MHC-I and initiate a response against cancer cells. The MHC-I molecule is often downregulated in most cancer cells [53]. Even though the reduced expression of MHC-I molecules in myeloma cells enables NK cells to detect them, an impaired response against the myeloma cells is present in most patients [50]. The impaired response is believed to be regulated through progressive alteration of the NK cell receptor ligands. For instance, a strong downregulation of NKG_2D, which grants protection against immunosurveillance and promotes myeloma cell survival in transgenic Vk*myc mice [54-50]. NK cells in MM tend to upregulate CD38, a multifunctional cell surface protein with enzymatic functions that induces NK cell activation which can be recognized by B and T cells [55]. Targeting CD38 with treatment would increase NK cell activation without B or T cell stimulation, thereby increasing the immune response against MM [56]. Several components of the immune system contribute to the survival of myeloma cells and therefore the progression of the disease. Combined impairment of the adaptive and innate immune response enables the myeloma cells to escape from the immunosurveillance and exploitation of the stromal leukocytes for multiple growth factors and cytokines (*figure 2*) [50].

Inflammation

An ongoing inflammation supplies the myeloma cells with multiple signaling mechanisms that drive MM progression. Previous studies have shown the influence of the stromal immune cells on the self-stimulating properties of myeloma cells that become accessible through inflammation [23]. An imbalanced T cell population combined with MPCs and other surrounding cells induce the secretion of a variety of cytokines. Consisting mainly out of pro-inflammatory cytokines but tumor-suppressive anti-inflammatory cytokines as well [23]. Multiple cytokines induce MPC proliferation and survival. The

diverse landscape creates a complicated network of inflammatory agents with dualistic abilities considering anti-tumor and pro-tumor activity. IL-1, IL-6, IL-17, and TNF are frequently referred to as the key cytokines that promote pro-tumor activity in MM [57]. A crucial factor for B cell differentiation is increased expression of IL-6, it also functions as an activator of JAK/STAT and RAS/MAPK which largely contribute to myeloma cell growth, differentiation and apoptosis inhibition [57].

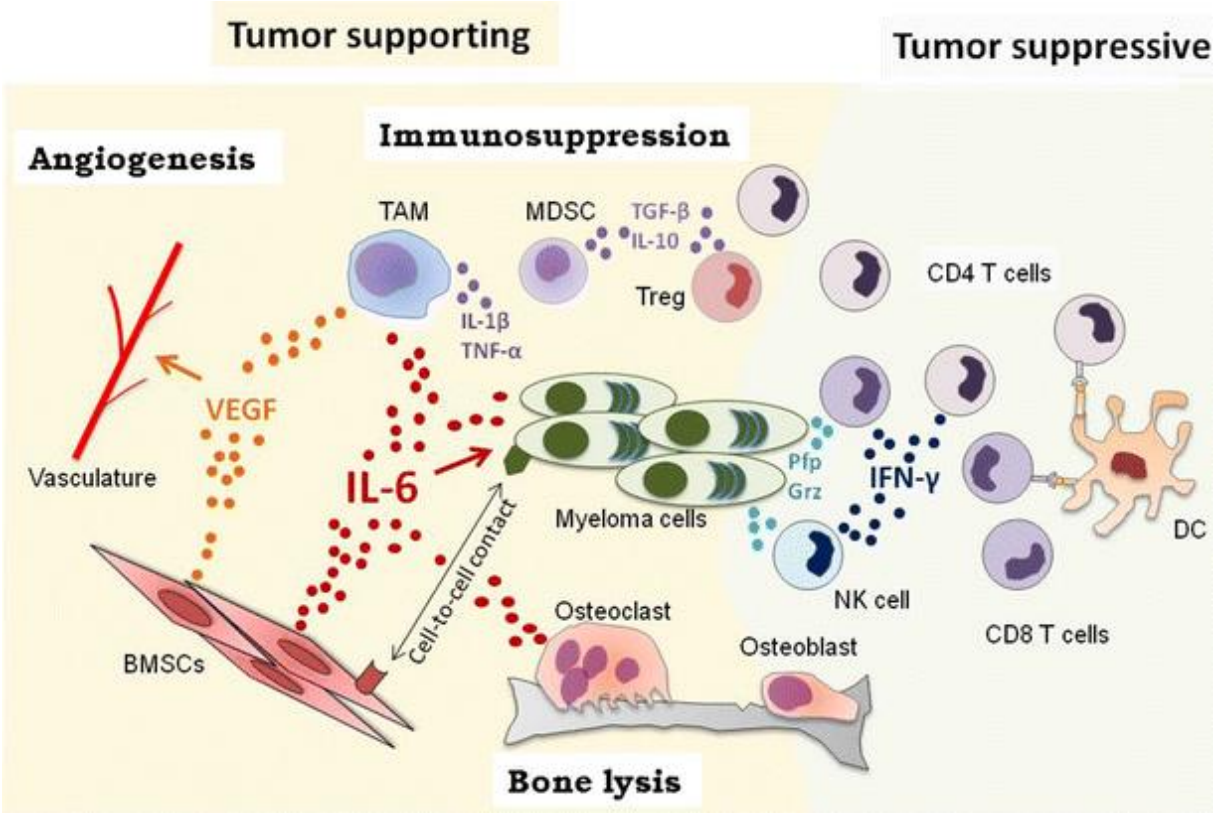

Figure 2: The role of the immune system and stromal cells in multiple myeloma [50].

Multiple cytokines induce an increase in IL-6, activated Th17 cells secrete IL-17 for example, which results in more IL-6 secretion from eosinophil granulocytes [9]. Thereby, contributing to systemic inflammation. It has been proven that commensal gut bacteria are involved in inflammation transgenic Vk*myc mice, which increase IL-17 levels and therefore supporting the progression of MM [9]. Additionally, obesity has been proven to be a risk factor in MM with upregulated levels of IL-10 and TNF-α [8]. Regulation of pro-inflammatory factors is vital for maintaining homeostasis within the immune system and enabling systemic recovery. There are several anti-inflammatory factors present within MM. However, not all share anti-tumor activity-related traits, thus creating controversial effects. The impaired regulation of cytokine secretion is believed to play a big role in the progression of MGUS to MM [11]. Tregs mainly secrete IL-10 which suppresses inflammation, however, this mechanism is impaired in MM and accordingly involved in pro-tumor activity [57]. IL-10 is arguably the most powerful anti-inflammatory cytokine that can be produced by a vast majority of the immune cells. On the contrary, IL-10 promotes the humoral immune responses and increases Ig production which is not desired for MM [58]. Finally, the role of Tregs is discussed while there exist conflicting reports on the functionality of Tregs in MM [57]. Although myeloma Tregs are believed to be functional, it is still questionable how effective their suppressive abilities remain after infiltration in the BMM. However, no correlation has yet been found between the role of Tregs and the progression from MGUS to MM [59].

The bone marrow microenvironment

The BMM & bone loss

The primary function of the microenvironment in the bone marrow is to regulate and support the production of blood cells to maintain homeostasis [60]. The microenvironment homes perivascular stromal cells that provide signaling for hematopoietic stem cells (HSCs). HSCs are multipotent cells that are able to differentiate in every type of blood cell. The HSCs inhabit the center of the bone marrow and interact with the microenvironment through signaling. In order to maintain a healthy hematopoietic system, the signaling molecules regulate the HSC quantity, self-renewal, trafficking and quiescence [60]. The multicellular structures in the bone marrow that support hematopoiesis form the BMM, are often referred to as a niche for blood cells. However, this important homeostasis-maintaining mechanism is susceptible for alterations and invasion in cancer. Dysregulations cause increased signaling and contribute to cancer cell proliferation and differentiation.

The stromal cells, systemic energy levels, inflammatory mediators, endocrine signaling, adhesion molecules and damage renewal, these factors define the BMM as a niche for myeloma cells [28]. Myeloma cells disrupt the systemic regulation of hematopoiesis, hence, the anemia and weariness [28]. The most notorious symptoms of MM are the lytic bone lesions, frequently visualized on X-rays or low-dose whole-body CT scans when patients suffer from fractured bones (*figure 3*)[20]. The lesions are due to consistent stimulation of osteoclasts, which persistently decrease the bone matrix in such a way that osteolysis occurs [36].

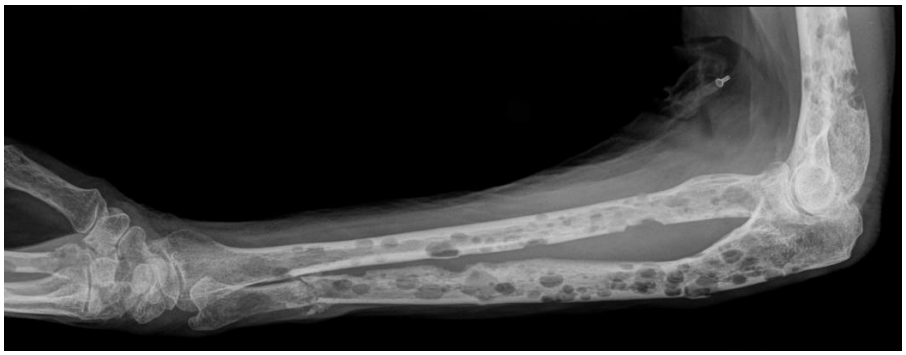

Figure 3: An X-ray image of a MM patient, showing lytic-bone lesions and a fracture in the underarm [1]

In healthy circumstances, the activation and proliferation of osteoclasts is regulated by stromal cells and osteoblasts in the bone marrow. The regulation is mediated through the RANK-L /RANK/osteoprotegerin (OPG) system. RANK is present on osteoclast progenitors and its activation will lead to osteoclast differentiation and maturation, which contributes to bone resorption [36-61]. Normally, RANK-L activity is physiologically antagonized by INFγ in order to prevent excessive bone destruction. In MM, the expression of RANK is dysregulated on osteoclast progenitors. Additionally, myeloma cells overexpress RANK-L, TNF-α, MIP-1α and downregulate the RANK-L decoy receptor, resulting in direct activation and formation of osteoclasts [62-36]. The inhibition of the RANK-L decoy receptor is believed to be a consequence of VLA4 and VCAM1 interaction. Causing elevated IL-6 and RANK-L levels but also decreased INFγ levels, which favor bone resorption [63-64].

Tumor sustainability

Tumor growth is often dependent on a constantly sufficient blood supply. The growth requires the formation of new blood vessels, which is accomplished through angiogenesis [27]. The stromal cells and myeloma cells in the BMM overexpress vascular endothelial growth factor (VEGF) that stimulates proliferation and chemotaxis in endothelial cells [51]. Furthermore, the BMM induces vasculogenesis, this process forms new blood vessels by recruiting endothelial progenitor cells surrounding the myeloma cells [31]. Additionally, the expression angiogenesis factors FGF-2, TNF-α, IL-6, IL-8, CXCL12 and several Notch family members are upregulated. As a result of myeloma cell interaction with bone marrow stromal cells, novel blood vessel formation is induced [65]. The formed blood vessels enable immune cell migration towards the BMM and supplementing protein arrival that support myeloma cell sustainability [31].

Mesenchymal stem cells (MSCs) are progenitors of multiple BMM cells, such as osteoblast, osteoclasts and adipocytes. MSCs have highly dynamic abilities including self-renewal, differentiation, cell-signaling, injury and tumor homing and immunomodulation [28-66]. The influence of stromal cells on the MSCs though molecular signaling is a determinant factor in pathological alteration of the MSCs. The myeloma cells are able to hijack the MSCs, thereby providing the cells with a positive-feedback loop of several tumor growth enhancing cytokines [67]. The role of pharmaceutical agents targeting MSCs are currently under investigation because of promising myeloma cell growth-inhibiting abilities [28].

Adipocytes arise from MSCs and serve as an energy stockpile in the bone marrow, it is the main cell type that is present in adipose tissue. The adipocytes in the BMM interact with myeloma cells through endocrine signaling and cytokines that support progression of the disease [28]. The secreted cytokines include TNFα, IL-6, IL-10 and C-reactive protein (CRP), CRP has been linked to activity of the immune system and may even serve as a biomarker for inflammation [68]. These findings suggest the involvement of adipocytes in myeloma cell survival by providing energy and growth factors derived from chronic inflammation. Additionally, adipocytes in the BMM contribute to chemotherapy resistance in myeloma cells through upregulation of autophagy, which suppresses caspase cleavage and thereby apoptosis [69]. Adipocytes secrete adipokines, adipokines are known to increase the expression of autophagy-proteins and might be suitable for a therapeutic target to encounter chemotherapy resistance [69].

Treating multiple myeloma

Standard treatment drug classes

Even though novel therapies developed for MM that considerably enhanced the quality of life and increased the survival rates in patients, the disease remains incurable. Relapse is frequently observed in patients and drug-resistant properties often emerge through an increasing mutational load and cross-talk with the BMM. In the last decade new therapeutic approaches have been combined to combat progression and to provide new insights, nevertheless, chemotherapy and steroidal treatments remain very effective for a majority of MM patients [70-28]. A widely used class of drugs against MM are proteasome inhibitors (PIs), blocking proteasome activity results in protein accumulation in myeloma cells, thereby inducing self-destruction. Bortezomib is regularly prescribed in MM for its apoptosis-inducing abilities, not only myeloma cells are targeted by Bortezomib, also osteoblast differentiation and osteoclast-apoptosis are increased by the drug [71]. Bortezomib has proven to be very promising in several treatments and can be combined aside other drugs, several novel PIs are currently under development in order to increase effectiveness and decrease peripheral neuropathy [72]. PIs are suitable for long-term treatment, however, the myeloma cells develop resistance over time. The resistance is believed to occur due to increased efflux through the P-gp transporter and changes in metabolic pathways of myeloma cells [73]. The resistant myeloma cells escape from the treatment and continuously expand until patients relapse fatally.

Treatment of the lytic-bone pains that most MM patients experience, can be accomplished through bisphosphonates. The class of drugs prevents bone-resorption by inhibiting osteoclast activity, this relieves the patient from excessive pain and enables conjugation with PIs [74-28]. Combined treatment models often include immunomodulatory drugs (IMiDs), monoclonal antibodies (mAbs) and corticosteroids such as dexamethasone and prednisolone to suppress inflammation. IMiDs affect MM locally and systemically, locally IMiDs like thalidomide, lenalidomide and pomalidomide enhance the susceptibility of myeloma cells to bortezomib and dexamethasone. Systemically they stimulate the anti-tumor response of the immune system and partially inhibit tumor-growth signaling from the BMM, computing patients to be less prone to relapse [75]. Despite the effective treatments, the efficacy of most drugs reduces after time until the treatment is no longer beneficial for the patient. Thus, implying for a preventive approach.

Transplantation

Autologous stem cell transplantation (ASCT)and chemotherapy are often integrated in the standard treatment plans of MM. ASCT can be described as a transplantation of hematopoietic cells that were subtracted from the bone marrow of the patient and placed back to after high-dose chemotherapy [76]. The treatment offers progression free survival and an increased overall survival rate for several years, therefore, providing an alternative for relapsed MM patients. Despite the beneficial survival rates ASCT offers, patients still have to be eligible for the treatment due to the physical impact. ASCT is a very intense treatment and is not suitable for every patient, which is understandable while the mean age of first diagnosed MM is 69-years [4]. Additionally, MM patient that underwent ASCT treatment have an increased risk for catching infections, which can be fatal if not treated properly [77]. A new insight in MM provided a possible addition to the ASCT treatment. Dysfunctional Dendritic cells (DCs) were subtracted alongside the hematopoietic stem cells from patients and enhanced to functional anti-tumor DCs. The enhanced DCs, in combination with CTLA-4 blockers to prevent T cell exhaustion, increased the effective immune response against MM [78]. For now, ASCT treatments remains an effective way to treat a majority of eligible patients, the treatment might be even more effective after infusing the enhanced DCs in patients to provide additional immunotherapy for patients. However, transplant recipients have an increased potential risk of death that is not

associated with their disease [76]. Secondary primary cancers, infections, cardiac events and other treatment-related hematological malignancies were the most commonly observed non-relapse mortality factors [79-76]. In short, ASCT can be beneficial for MM patients for a limited timeframe, but it comes with dangerous risks and is considered to be a very exhausting treatment.

Novel therapies

A major arising class of drugs are mAbs, antibodies have immunomodulating properties that enable a targeted immune response against MM and alteration of the stromal immune cells in the BMM. A distinguished antibody is daratumumab, it binds to the cell-surface protein CD38 which is highly expressed on myeloma cells, making it a myeloma-specific executioner [56]. The discovery of various cell-surface proteins that are vastly expressed by myeloma cells has led to an increase in approved mAbs against MM. Indatuximab ravtansine is in pre-clinical trials after being successful in MM treatment combined with radiotherapy in animal models. The drug targets CD138 which is expressed in approximately 95% of myeloma cells [80]. Several other mAb treatments are momently under investigation, clinical trials show promising effects, especially when mAbs are combined with other drugs, thereby providing an effective treatment for MM patients with limited unwanted side-effects. A downside of mAb treatments is the immunological background of the patients. Low amounts of active immune cells in the BMM will affect the affinity for the treatment, which results in reduced response rates (*figure 4*). Furthermore, the anti-tumor effect of antibodies comes to a hold after a certain time, possibly due to clonal evolution of the myeloma cells. However, it remains poorly understood how this resistance occurs.

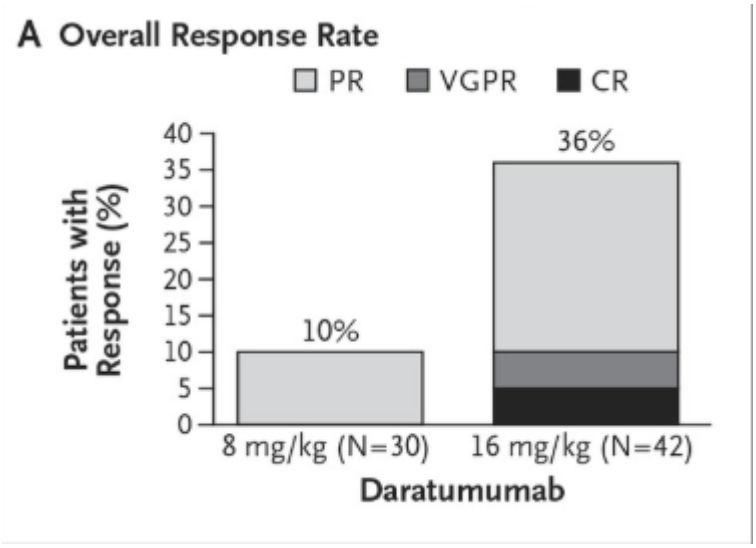

Figure 4: The response rates of Daratumumab, complete response (CR), very good partial response (VGPR) and partial response (PR) [56].

Currently multiple novel drug classes are under investigation, designed to combat the progression of MM. Histone deacetylase inhibitors (HDACi) are analyzed for their transcription-suppressing abilities and combinable abilities with PIs and IMiDs, resulting in constrained cell-growth and apoptosis-stimulation in myeloma cells [81-82]. Furthermore, a promising treatment for encountering MM is Chimeric antigen receptor (CAR) T cell therapy. CAR T cells are cytotoxic T lymphocytes supplied with a tumor-specific antibody, which enables highly accurate recognition of the tumor cells and myeloma cell death [83]. Previous trials with CAR T cell therapy targeting B cell maturation antigen, which is commonly expressed in myeloma cells, showed promising results and an overall response rate of 81% [84]. Despite the high response rates, some severe side effect were observed, such as

neurotoxicity and cytokine release syndrome. However, combining the therapy with anti-IL-6 mAbs significantly reduced the unwanted aftereffects, making it a more suitable treatment [83]. A recently discovered CAR T cell target is integrin β7, which is expressed on myeloma cells and normal lymphocytes. N. Hosen et al, identified a highly specific mAb against integrin β7 named MMG49. MMG49 binds to the N-terminal on the β7-chain, on normal lymphocytes this binding-area is inaccessible for the antibody. Myeloma cells show an altered conformation, thus, offering an accessible binding area for the mAb that is highly specific for MM cells [85]. Equipping CAR T cells with MMG49 offers a very promising prospect for novel treatment, while it ignores normal hematopoietic cells but targets myeloma cells and precursor-myeloma cells [83]. Still, the realization of clinical CAR T cell therapy is not achievable yet. Considering the clinical trials that have to performed to evaluate toxicity and efficacy. Finally, the treatment would be unavailable for a majority of patients, due to the high costs [11].

How the progression to multiple myeloma can prevented by targeting cell-interactions in the bone marrow microenvironment

Although the transition of MGUS to MM has been intensively studied in the past decade. It still remains mysterious how the progression occurs, considering the anergic ability of the disease preventing it from turning into a malignant variant. Current and novel treatments might be effective and promising, but they are directed at treating MM symptoms. However, patients still encounter those treatment-related neurotoxic-symptoms and only increase their life-span by a limited amount of years. Moreover, the prospects after being diagnosed with MM do not seem promising at all.

Preventing the conversion of MGUS to MM could be a desirable approach for curing the disease, since all newly discovered therapeutic targets might form a remedy against progression. Targeting the main signaling stromal cells that are located in the BMM, could reduce MPC differentiation in MGUS patients. Myeloid-derived suppressor cells (MDSCs) are progenitor cells of macrophages, granulocytes and dendritic cells under healthy circumstances [86]. During the progression of MM, MDSCs differentiation is inhibited which results in accumulation in the BMM [87]. The dysfunctional MDSCs stimulate tumor growth through angiogenesis and inflammation, but also by inhibiting T cell receptor signaling directed against tumor cells [50]. Depletion of MDSCs with PDE5 inhibitors in a mice study revealed tumor growth inhibition, suggesting that targeting MDSCs might be valuable for disease prevention [88-87]. IMiDs already enable immunomodulation of the stromal cells and boost myeloma cell clearance. Despite their abilities of clearing myeloma cells, the IMiDs are not suitable for preventive treatment. Treating patients with high dosage can lead to acquired von Willebrand syndrome, the syndrome is a clotting disorder that causes bleedings [89]. However, lose-dose IMiD treatment alongside PD-1/PD-L1 inhibitor mAbs have been proven to induce NK cell activation and improve cytotoxic T lymphocyte functions [87]. Further research might reveal preventive abilities of low-dose treatment. An additional way of interfering in the ongoing inflammation would reduce cytokines, growth-factors and offers a recovery-window for functional immune cells in the BMM. Thus, instructing the immune system to recognize the MPCs in MGUS patients would support prevention and clearance from the BMM. Guiding the immune system can be accomplished by stimulating dendritic cells, by targeting Toll-like receptors (TLRs) with CpG oligodeoxynucleotide [90]. Moreover, MM cell growth can be inhibited with TLR-9 agonist C792 (*figure 5*), which activate the TLR9/MyD88 signaling axis in DCs [91]. The signaling axis induces the secretion of interferons that stimulate the anti-tumor response [87]. Therefore, these findings suggest several possibilities for clinical trials that might be valuable for preventive treatment.

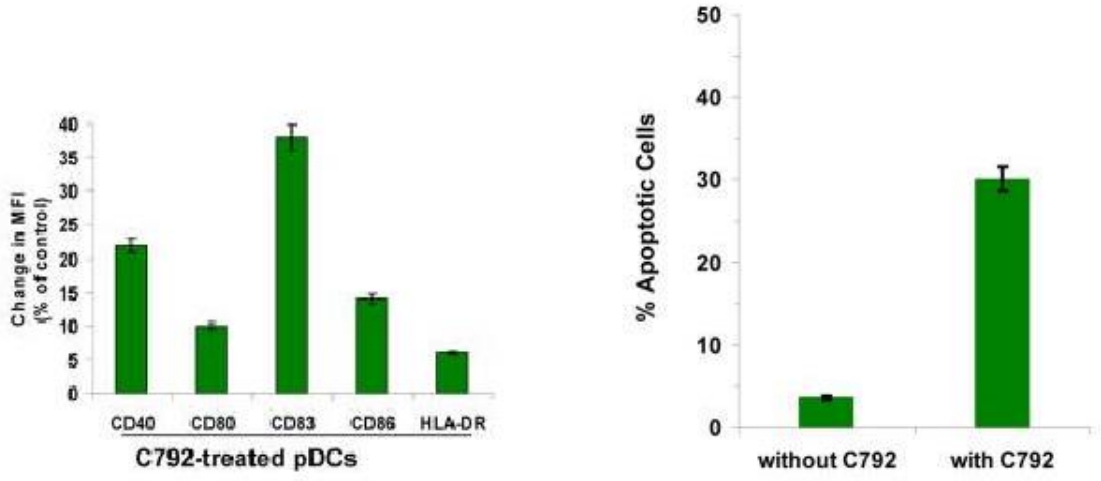

Figure 5: Left graph: MM-patient bone marrow-plasmacytoid dendritic cells ($CD123^+/BDCA\text{-}2^+/HLA\text{-}DR^+/CD11c^-$) were cultured in presence or absence of C792 for 12h. Fluorescent antibodies were used to stain CD40. CD80, CD83, CD86 or HLA-DR, followed by flow cytometry analysis. The left bar graph shows the change of co-stimulatory cell-surface molecules that indicate activation of the immune system. The right bar graph displays the amount of apoptosis in MM.1S cells after 12h treatment with C792 and plasmacytoid dendritic cells [91].

Furthermore, sharp observation of the BMM in MGUS during the transition to SMM is necessary for locating a suitable intervention time-frame. The preventive treatment would induce an early enhanced immune response. Additionally, crucial elements for disease progression would become unavailable for MPCs if NF-κB,IL-1, IL-6, TGF-β and VEGF expression levels were diminished during MGUS [92]. These findings suggest that the cell-interactions in the BMM can be targeted by treatment to prevent progression.

To study the effects of pharmaceutical agents and disease progression, an immunodeficient mouse-model that replicates the human BMM niche was developed by Richard Groen et al [93]. The model offers a new insight on the interaction of the BMM and myeloma cells, perhaps, the model can even be adjusted to a disease state that resembles the progression to SMM. Providing such a model would raise preventive research possibilities. Yet, the aging effect of the disease is very hard to imitate in mouse models due to age limitations of mice. Surprisingly, previous clinical trials proved that antagonizing IL-6 in MM has little effect on slowing down the disease [94]. Thus, emphasizing the influence of BMM interaction with myeloma cells. Antagonizing signaling molecules in a novel transition mouse-model might elucidate the transitional effect and the discovery of novel pleiotropic signaling systems that remain hidden in the BMM.

Discussion

A heterogeneous landscape with difficulties

The heterogeneous tumor landscape remains one of the biggest hurdles in MM, the origin of the sudden progression can be traced back to the BMM where uncontrolled growth is achievable. Interfering with the aberrations and the dysregulations in immunology, vascular, epigenetic and metabolic factors at an early state of the disease could be vital. Preventing the transition from MGUS or SMM would circumvent the establishment of MM. Thereby saving patients from bone-injury and very expensive treatment of an estimated 20.000$ per month, that increase the life expectancy by a couple of years but do have neurotoxic side-effects [95]. The identification of novel therapeutic targets over the last decade gave rise to novel treatment plans and insights for preventive measures. The cell-interactions in the BMM resemble a prospective target to realize prevention. Several scientific findings on immune modulations, such as DC stimulation and inhibition of MDSC accumulation, show promising results against myeloma cell growth. Further studies on the rehabilitation of the immune system during MGUS and SMM might improve these potential preventive-treatments. In order to expand the current knowledge about the capability of combining treatments for prevention, more preclinical trial have to be performed. Testing drug-conjugates in a model that represents the BMM during MGUS, would provide increased and novel options for preclinical trials. Additionally, researchers do have to keep in mind that MGUS never proceeds to MM in the majority of described cases [15]. This is a major obstacle for preventive treatment, because it would require very intensive screening and surveillance of MGUS patients. Else, the treatment might impair normal hematopoiesis while progression to MM would have never happened.

Concluding remarks

The current treatment models show remarkable improvements over the past decade, patients retain a better quality of life with extended years to live and. However, the burden patients have to carry due to treatment and relapses indicates the eagerness of preventing MM. The BMM homes a variety of signaling cells that contribute to MM progression by inducing angiogenesis, inflammation and supplementation of growth-factors. Interfering with these interactions at an early time point halts the MPCs from proliferating and growing. Prevention requires great understanding on the progression of the disease and the cell-interactions in the bone marrow. Combining multiple therapies that target the these interactions appear to be suitable for prevention. Yet, further studies on drug-conjugates and selecting the most beneficial treatment-timeframe are required to realize prevention.

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