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Adipose, Bone and Myeloma: Contributions from the Microenvironment

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Abstract

Researchers globally are working towards finding a cure for multiple myeloma (MM), a destructive blood cancer diagnosed yearly in ~750,000 people worldwide [1]. Although MM targets multiple organ systems, it is the devastating skeletal destruction experienced by over 90% of patients that often most severely impacts patient morbidity, pain, and quality of life. Preventing bone disease is therefore a priority in MM treatment, and understanding how and why myeloma cells target the bone marrow (BM) is fundamental to this process. This review focuses on a key area of MM research: the contributions of the bone microenvironment to disease origins, progression, and drug resistance. We describe some of the key cell types in the BM niche: osteoclasts, osteoblasts, osteocytes, adipocytes and mesenchymal stem cells. We then focus on how these key cellular players are, or could be, regulating a range of disease-related processes spanning MM growth, drug resistance, and bone disease (including osteolysis, fracture, and hypercalcemia). We summarize the literature regarding MM-bone cell and MM-adipocyte relationships and subsequent phenotypic changes or adaptations in MM cells, with the aim of providing a deeper understanding of how myeloma cells grow in the skeleton to cause bone destruction. We identify avenues and therapies that intervene in these networks to stop tumor growth and/or induce bone regeneration. Overall, we aim to illustrate how novel therapeutic target molecules, proteins, and cellular mediators may offer new avenues to attack this disease while reviewing currently utilized therapies.

1. Introduction

To understand opportunities for therapeutic interventions which target the bone environment of patients with MM, it is essential to understand the role of the bone marrow (BM) niche in

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MM, the key niche components, and how they communicate with tumor cells. Although numerous cell populations, including immune cells, reside in the BM microenvironment (Figure 1), this review will specifically cover the roles of osteoblasts, osteoclasts, osteocytes, bone marrow-derived mesenchymal stem cells (MSCs), and bone marrow adipocytes, in myeloma and myeloma-associated bone disease. A major goal of current research is to identify new mechanisms for targeting local BM cells, or their derivatives, to influence the disease course of MM, and the clinical utility of this approach holds immense promise. An example of this is demonstrated by the widespread success of anti-resorptive therapies such as bisphosphonates in MM patients. These osteoclast-inhibiting therapies work by blocking osteoclast bone resorption, which has been shown to reduce skeletal-related events (SREs) in patients with bone lesions from MM [2]. Though the targeting of osteoblasts, osteocytes, and adipocytes also holds untapped potential, how to do this and what biochemical pathways to target, remain unanswered questions in MM. However, it is becoming increasingly clear that MM cells are dependent on the BM for survival and drug resistance, and that interference in this support will be made more effective by identifying the key cells, proteins, and pathways that make this niche so hospitable to MM cells. Importantly, targeting the cells of the bone microenvironment may be a more broadly-applicable therapy, as many of the dysfunctional attributes of the marrow appear independent of the mutational landscape that is intrinsic to the tumor cells. Furthermore, much of the morbidity and mortality caused by MM is related to cancer-induced bone disease, and associated hypercalcemia, bone fracture, spinal cord compression, and hindered immune function. Through targeting the bone microenvironment specifically, we may also combat MM-bone disease by preventing further bone loss and rebuilding lost bone, repairing lesions, and preventing fractures. Through an improved understanding of the relationships between these BM and tumor cells, researchers will identify new molecular pathways to target with new or repurposed therapeutics, and discover new methods for disease prevention or improved combinatorial treatments.

2. Understanding the Bone Marrow Niche Cellular Components

Osteoclasts, Osteoblasts, and Osteocytes

Bone is a complex organ made up primarily of three cell types, osteoclasts, osteoblasts, and osteocytes, which signal to each other to help retain the equilibrium between bone resorption and formation essential for bone health (Figure 1a). Osteoblasts are bone-forming cells that reside on the endosteal, periosteal, and trabecular surfaces of bone. Osteoclasts also reside along these bone edges, adjacent to osteoblasts, and are responsible for bone resorption. Osteocytes are terminally differentiated osteoblasts which are embedded deep within bone matrix. Here, osteocytes signal to osteoblasts and osteoclasts to control bone mass, directing them to either make or resorb bone depending on physical, endocrine, paracrine and autocrine signals. These three main cell types together make up what is known as the “BMU”—the basic multicellular unit—that is responsible for maintaining skeletal mass and integrity and healing bones after fracture. These cells also form the endosteal niche which is often referred to in the context of hematopoietic stem cell (HSC) renewal [3]. These endosteal niche components are key players in skeletal response to diseases such as myeloma. Although not covered in this review, the bone marrow niche also interacts with multiple other bone resident cells including immune cells and neurons.

Osteoclasts are derived from HSCs differentiated down the macrophage pathway and produce acid and collagenases to break down bone matrix. Osteoclasts contain a high number of vacuoles, vesicles, and lysosomes which store enzymes such as tartrate resistant acid phosphatase (TRAP) and cathepsin k, a collagen protease, essential for bone resorption. These multinucleated cells secrete their acidic, collagenase products into resorption pits, protecting the nearby BM cells from these harsh conditions through a sealing zone. Within this sealed-off space, osteoclasts have a high cell membrane surface area termed a ruffled border, which facilitates a high secretion and uptake rate. Osteoclasts differentiate from pre-osteoclasts through receptor activator of nuclear factor kappa-B (RANK) and RANK ligand (RANKL) signaling; RANKL is produced primarily by osteoblasts and osteocytes [4].

The second cell type discussed, osteoblasts, are singly-nucleated cells that work in concert to form new bone along the border of mineralized bone matrices. These specialized, cuboidal cells are derived from MSCs and produce osteogenic cytokines and bone matrix elements including very dense collagen (mostly type I), and smaller noncollagenous proteins, including osteocalcin and osteopontin. Once this organic matrix (osteoid) is laid down, osteoblasts mineralize it with mechanically robust inorganic components (hydroxyapatite, calcium carbonate, and calcium phosphate). Upon cessation of mineral deposition and proliferation, an osteoblast can either be embedded into the bone matrix by neighboring osteoblasts, transforming it into an osteocyte, or it can become a quiescent bone-lining cell. Bone-lining cells, only distinguishable from osteoblasts through morphology and function, are thought to be a source of osteoblast progenitors and line the canopy often described in association with BMUs [5]. To date, distinct molecular profiles of these quiescent bone lining cells have not been identified, however they are highly abundant in bone and therefore important to consider in the setting of myeloma.

Lastly, as osteocytes compose 90% to 95% of all bone cells in adult bone [6] and are major regulators and coordinators of bone formation and resorption, their contributions to myeloma-induced bone disease are likely very important. Osteocytes are incredibly complex mechanosensing cells, able to detect and respond to numerous soluble mediators and mechanical signals in their environment. They sense physical forces by detecting fluid flow on their cilia and other cell processes, stimulating them to signal to local bone cells to control bone homeostasis. Signaling between osteocytes themselves and to endothelial cells throughout the bone matrix is achieved through microvesicles, gap-junctions at the ends of long cytoplasmic projections, and factors secreted directly into the canalicular fluid within the lacunae-canalliculi network. Osteocytes are also capable of remodeling the perilacunar/canalicular matrix, forming and even resorbing bone, in response to local signals [6] that alter their expression of a number of proteins. One of these key proteins is sclerostin, a Wnt inhibitor that blocks osteoblast differentiation and potentially accelerates osteoclastogenesis and adipogenesis in the bone marrow [7]. Others are DMP-1, RANKL, matrix extracellular phosphoglycoprotein (MEPE), and other signaling factors that regulate bone formation and resorption [6]. Changes in protein expression cause signaling cascades through dendritic projections directly connected to bone-lining cells, osteoclasts, osteoblasts, myeloma cells, and, potentially, bone-lining “osteoadipocytes,” a term coined by Dr. Clifford Rosen at the 2016 Keystone Adipose Tissue meeting [8].

Marrow adipocytes

Marrow adipocytes reside along the endosteal surface and throughout the BM with different frequency in distal versus proximal BM cavities in long bones. Marrow adipocytes have long been ignored as they are often viewed as inert “filler cells” rather than the metabolically active and communicative cells they are [3, 9]. Recent work has demonstrated a more complex role for bone marrow adipose tissue (MAT) within the BM niche [10] than previously acknowledged. Exciting new findings demonstrate two distinct types of marrow MAT: *constitutive MAT*, found in the distal tibia and tail of rodents and formed at a young age, and *regulated MAT*, which appears upon aging in proximal femora and vertebrae in close proximity to hematopoietic elements and trabecular bone [11]. Constitutive MAT (cMAT) volume, measured by MRI in humans and osmium microCT in rodents, appears relatively static and may negatively impact hematopoiesis, possibly by maintaining HSCs in a quiescent state [11, 12]. Conversely, regulated MAT (rMAT), as the name suggests, can be regulated or modulated by influences such as age, diet, pharmaceuticals, or other endocrine and paracrine influences [13]. Elevation in rMAT has been correlated in human studies with decreases in cortical bone, bone volume, bone formation rate, and occurrence of osteoporosis and osteopenia [11].

The process of adipogenesis and osteogenesis has traditionally been considered mutually exclusive, such that the transcription factors and pathways that induce osteoblastogenesis inhibit adipogenesis and *vice versa* [3]. However, significant lineage plasticity exists between osteoblasts and adipocytes, which share a common progenitor that further complicates dissecting the relationship between these two cell types in healthy and cancer-containing bone marrow [14]. Cell-lineage tracing experiments demonstrate that BM adipocytes, like osteoblasts, are derived from osterix-positive cells and are more closely related to osteoblasts and chondrocytes than are peripheral white adipocytes [15]. However, recent evidence suggests that BM adipocytes may also derive from a progenitor cell that is distinct from the progenitor for osteoblasts, chondroblasts, and other BM stromal cells [16]. Moreover, a plasticity between BM adipocytes and osteoblasts is also evident in their ability to potentially transdifferentiate and “jump the track” between these two cell maturation fates after initiating differentiation [17]. These data emphasizes the need for more lineage tracing studies of the BM adipocyte to improve our understanding of this unique cell type.

Importantly, there appears to be a reciprocal relationship between MAT and bone formation in some physiological and pathophysiological conditions [11]. This growing evidence linking MAT with low bone density supports the concept of BM adipocytes as a potential cause of the underlying pathology of bone loss in MM. However, other studies have found that increasing cancellous bone volume per total volume (BV/TV), observed in rat caudal vertebrae in relation to the distal direction, also correlates with increased cMAT (yellow marrow volume), indicating a direct linear, rather than inverse, relationship between bone and adipose volumes [18]. Therefore, deciphering the direction of cause and effect with MAT and bone loss is challenging as the relationship between BM adipocytes and bone cells remains elusive and becomes even more complex when assessed in the presence of myeloma cells. It is also clear that bone has a complicated, non-linear, genotype-dependent relationship with energy metabolism and MAT [19, 20]. Mice fed a high fat diet and humans

with increased visceral adiposity also have an accompanying increase in MAT, providing a potential mechanism whereby obesity increases the risk for osteoporotic fractures due to increased MAT [21], although no bone changes were observed in this study, perhaps due to the short time course of observation. New data reveal that common genetic and environmental factors are shared between obesity and osteoporosis, suggesting that excessive adipose may not protect against osteoporosis but actually accelerate it [22, 23]. OVX-induced osteoporosis studies have demonstrated that bone loss can occur prior to increases in MAT [24]. Exercise has been shown to significantly suppress MAT volume and induce bone formation in certain mouse models, suggesting that a double-edged sword may be strengthening bones and decreasing MAT in MGUS or MM patients via diet and exercise to improve bone outcomes [25].

Marrow adipocytes have properties which make them both distinct from and similar to adipocytes in other depots. MAT, often termed yellow adipose tissue, has gene expression patterns that overlap with both white and brown fat, highlighting its uniqueness [23]. Moreover, MAT expression of certain proteins (e.g. Dio2, PGC1 α , and FOXC2) [23] is much higher than white adipose tissue (WAT) expression and in certain conditions, such as in caloric restriction (starvation or anorexia), WAT and MAT respond in opposite manners: while WAT decreases, MAT increases [26]. Brown adipose tissue (BAT), WAT and MAT also have been found to express very different levels of numerous adipokines [27]. In addition to the unique physiology of MAT, the close physical proximity of MAT and MM cells suggests unique, bidirectional signaling between MM cells and BM adipocytes unlike signaling between WAT and MM cells.

Yet similarities between adipose depots cannot be ignored. For example, in obesity, both WAT and MAT display marked increase in the size and number of adipocytes [28]. Recent data also suggests that systemic white adipocyte populations, typically thought to derive from tissue resident mesenchymal progenitors, are actually comprised of cells that derive from the marrow (up to 35%), meaning that WAT and MAT may not be completely distinct depots [29]. MAT, WAT, and BAT-derived adipocytes also produce relatively similar amounts of the anti-inflammatory protein adiponectin, which functions to switch macrophage polarization from M1 to M2, thereby attenuating chronic inflammation [23]. It is important to note that MAT, and MAT responses to diet, drugs (e.g., rosiglitazone), cold exposure/thermoneutrality, unloading, and other environmental stimuli, have significant differences based on age, species, strain (eg. C57BL/6J vs C3H), sex and anatomical location [19, 21, 26, 30–32]. Moreover, many of the experiments characterizing MAT have not been done systematically across this spectrum of experimental conditions, and our understanding of human MAT is also relatively underdeveloped, in part due to the technical challenges of accessing and analyzing this tissue, and also due to previous omissions of this depot in research [33]. Therefore, before interpreting MAT effects on tumors, it may prove useful to comprehensively characterize MAT in humans and mice, to avoid misinterpreting results in the future based on incorrect assumptions regarding its function and lineage.

In summary, MAT tissue is now being recognized as a unique type of adipose, with a distinctive phenotype, response to stress, and physiological role. Even within the BM, it is clear that different regions of adipose respond differently to systemic energy levels and drug

treatments. These cells are now being explored for their contributions to health and disease through adipokine or lipid secretion and metabolic influences. A greater understanding of these special cells will likely expose new vulnerabilities in targeting cancers or other diseases of the BM.

Bone Marrow-derived Mesenchymal Stem Cells (BM-MSCs)

Adult, human MSCs are defined as cells which have the properties of adherence to plastic, expression of cell surface markers including CD29, CD44, CD90, CD49a–f, CD51, CD73 (SH3), CD105 (SH2), CD106, CD166, and Stro-1, and lack of expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR surface molecules, according to the International Society of Cellular Therapy (ISCT) [34]. Bone marrow-derived mesenchymal stem cells (BM-MSCs) are multipotent cells that are able to self-renew or differentiate down diverse lineages (chondrogenic, adipogenic and osteogenic) [35], and have more recently been observed to differentiate into other mesodermal cell types like skeletal muscle precursors and cardiomyocytes [36]. BM-MSCs also support the hematopoietic niche, as we recently reviewed [3].

Endosteal and Perivascular Niche

It has become clear, due to better technology and accumulated knowledge within the field, that the BM is not one niche but rather a compilation of multiple micro-niches, both creating and created by chemotactic gradients and distinct cell populations. These niches induce different responses in HSCs, including homing or mobilization, self-renewal, quiescence, or tightly-controlled lineage commitment differentiation. Two of the most prominent niches are the endosteal bone niche, which lines the bone surface and consists of flat, bone-lining cells, osteoblasts and osteoclasts, and the perivascular niche, the space encompassing the vasculature within the marrow. Evidence suggests that long-term, quiescent HSCs require and prefer the endosteal niche for their self-maintenance rather than the perivascular niche, due in part to different oxygen tensions and cell populations [37]. More differentiated cells, on the other hand, tend to occur at higher frequencies in central perivascular regions. Cell-cell signaling pathways that are the major mediators of HSC maintenance and homing to the BM include CXCL12/CXCR4, Jagged-Notch and angiopoietin-1-Tie2 [3]. Within the endosteal bone niche, osteoblasts were previously reported to retain HSCs in a quiescent state through expression of molecules such as osteopontin, angiopoietin-1 and thrombopoietin [38–40]. However, recent studies found no significant association between osteoblasts and HSCs but rather found that arteriolar niches may be responsible for maintaining HSC quiescence [41]. These data argue that perivascular niches, rather than osteoblasts, may directly contribute to HSC maintenance.

Perivascular niches, essential for gas exchange, nutrient delivery and waste removal in the BM, are composed of branching vessels that direct blood flow through the BM. Oxygenated blood enters the long bones through cortical bones through radial arteries and travels to up and down the central artery of the marrow [42]. This blood then diverges through radial arteries and into venous sinuses, which are thin-walled vessels consisting of a single layer of flat endothelial cells with little to no basement membrane. Venous sinuses are permeable for gas and small protein diffusion, and have weakly bound endothelial cells to allow for cell

mobility (intravasation and extravasation). Blood then collects from these capillaries, drains back into the central vein, leaves the marrow through nutrient veins through the cortex, and is pumped back to the heart and lungs [42]. Nestin-expressing MSCs have been found to preferentially localize along arterioles, and are an important component of the HSC perivascular niche through their expression of CXCL12 [43, 44]. Endothelial cells also modulate HSC function via Notch and other signaling pathways [45]. The cell surface markers CD150 and CD48, lymphocytic signaling and activation molecules, also appear important for HSC homing to sinusoidal blood vessels [46]. Interestingly, these HSC quiescence-inducing niches also provide the perfect environment for bone resident tumor cells such as prostate cancer cells, that can hijack and compete with HSCs for their niche [47]. The characteristics of these niches have been reviewed in-depth by Seshadri et al. and Yu et al. [45, 48]. In sum, although further clarification of the endosteal and perivascular niche components is necessary to understand HSC homing and function, it is clear that these niches are crucial for HSC function and may be important microenvironmental targets in MM.

3. Myeloma Interaction with BM elements

Osteoclasts, Osteoblasts, and Osteocytes

Cells in the bone microenvironment are a dynamic, diverse set of signaling nodes that display pro-tumorigenic or anti-tumorigenic effects, depending on cell and tumor type (Figure 1b). Osteoclasts and MM cells are involved in a feed-forward relationship whereby myeloma cells activate osteoclastogenesis and increase osteoclast activity (Figure 2), which leads to further tumor growth. MM cells increase local RANKL levels or inhibit the production of the RANKL decoy osteoprotegerin (OPG), either indirectly through changing osteoblast expression of these molecules, or by direct production of RANKL. Macrophage inflammatory protein (MIP)-1 α and MIP-1 β are also secreted by myeloma cells and play a major role in accelerating bone resorption [49, 50]. MM cell capacity for production of these molecules directly correlates with their ability to induce lytic bone lesions [51]. Myeloma-derived IL-3 and IL-6 also contribute to osteoclast activation. In turn, osteoclastic resorption leads to the release of matrix-bound factors which further stimulate tumor growth, thereby earning this process the name “the vicious cycle” [52]. Recently, osteoclast activity was directly linked to activation of dormant myeloma cells in bone, suggesting that osteoclasts may not only play a role in late stage vicious cycle remodeling but also in initiating tumor growth in bone or disease relapse [53]. Osteoclasts in general are thus considered to be tumor-supportive.

Compared to osteoclasts, the effects of osteoblasts and osteocytes on MM cells are less well defined (Figure 3). As with MM-MSCs [54], MM patient bone cells are abnormal: osteoblast proliferation and differentiation is inhibited [55], osteocyte numbers and lifespan are decreased [56], and osteocyte expression of osteoclastogenic cytokines is elevated [57]. MM cells inhibit osteoblast activity and new bone growth by secreting molecules such as Dickkopf-related protein 1 (DKK1), the soluble Wnt inhibitor secreted Frizzled-related protein (sFRP)-2, and TGF β /TSP1, as well as inducing osteocyte overexpression of sclerostin (SOST) [8, 58]. Unlike MM-supportive MSCs [54, 59, 60], osteoblasts appear to

suppress MM progression by inducing quiescence or apoptosis [53]. In one study, the pre-osteoblastic MC3T3-E1 cell line and differentiated, mineralized BM-MSCs induced MM cell apoptosis and cell cycle arrest [61], perhaps acting through molecules such as decorin [62]. Interfering with cell-cell interactions between osteoblasts and tumor cells has also been shown to impair tumor engraftment [60]. Osteoblasts can also have anti-tumor effects in hematological malignancies without osteolytic lesions, suggesting that they may have direct or systemic effects independent of the vicious cycle [63]. In addition, we and others have shown that quiescent MM cells prefer to reside in the endosteal/osteoblastic regions of the BM versus the vascular regions *in vivo*, indicating that osteoblasts may induce MM dormancy [53, 64]. However, inconsistencies in findings and mechanistic unknowns persist in this field [65]. For example, osteoblasts have been found to be supportive or inhibitory for MM depending on the MM patient cell source [66, 67]. Additionally, osteoanabolic agents have yielded conflicting conclusions in regards to MM cell growth, with *in vivo* and clinical efficacy dependent on model system and treatment used [65, 68]. Overall, further research is required to determine how best to target osteoblasts to alter tumor cell engraftment, survival, dormancy, and growth. Still, targeting the osteoblast represents an exciting direction for potential advances in combating both bone disease and tumor growth [52].

Osteocytes are undoubtedly critical players in MM, but their specific roles in osteolysis and disease progression are largely unexplored. Recent *in vivo* studies demonstrate increased osteocyte apoptosis and sclerostin expression in response to the presence of myeloma cells [8, 69]. Further *in vitro* studies confirmed that direct contact with MM cells induces increased expression of sclerostin in osteocytes thereby reducing Wnt signaling and subsequent inhibition of osteoblast differentiation [8]. The studies also observed that direct contact between osteocytes and MM cells reciprocally activated Notch signaling and increased Notch receptor expression, particularly Notch 3 and 4, which stimulated growth of MM cells. This work suggests novel targeting of bidirectional Notch signaling using receptor blockade may inhibit osteocyte MM supportive interactions, representing a potentially promising treatment strategy in MM [8], however this awaits *in vivo* confirmation with follow up studies.

Epidemiological data also suggest an inhibitory role of osteoblast-lineage cells on MM. Skeletal microstructural changes have been identified, along with elevated DKK1 and MIP-1 α levels, in MGUS patients [70]. Low bone mineral density, increased fractures, and osteoporosis correlate with MGUS [71], suggesting that dysfunctional bone cells could not only result from, but also contribute to MM. Much of the biological underpinnings of osteoblast or osteocyte effects on MM remain unknown; future work should aim to clarify the mechanistic basis behind the actions of these cells, to harness the potential of osteoblastic mediators to advance progress towards a cure for MM.

Understanding how different niches may differentially affect MM cell homing, engraftment, colonization, quiescence, drug-resistance, and disease relapse would provide researchers with a new perspective on ways to target MM. The current compilation of research suggests, that the endosteal niche, lined with osteoblasts, may be the niche where MM cells are protected in a quiescent state and able to resist chemotherapies [52, 53]. However, the switch

that reactivates these tumor cells to grow after decades of peaceful cell cycle arrest would provide an important novel prognostic biomarker and target if it could be elucidated.

Adipocytes

The nature of interactions between marrow adipocytes and myeloma cells is in the process of being revealed; accumulating data demonstrate that MAT may afford a protective environment for MM cells (Figure 4) through direct or indirect mechanisms, or may combat MM through expression of molecules such as adiponectin. *In vitro* experiments have demonstrated a role for MAT in increasing MM cell proliferation, potentially mediated by leptin or other adipokines, and protecting MM cells from chemotherapy-induced apoptosis by inducing autophagy [72, 73]. *In vivo* data also suggests that BM adipocytes play a large role in decreasing the efficacy of chemotherapies in MM [73]. Further, elevated MAT has been found to disrupt hematopoiesis and undermine the viability and fate selection of HSCs, which then leads to disrupted immune function. Suffocating the marrow cavity with MAT also has important consequences for individual cell types (osteoblasts, osteoclasts, mesenchymal cells, and endothelial cells) and their ability to interact normally with each other, along with the process of hematopoiesis. BM adipocytes serve as energy (lipid) reserves and express and secrete specific endocrine signaling molecules, including adipokines and growth factors (leptin, TNF α , MCP-1, and insulin), which may promote myelomagenesis by enhancing tumor growth or development [74]. However, a large prospective trial found no association between leptin levels and MM risk, but instead found a significant inverse correlation between adiponectin and MM [75]. A study from this year by Hofmann et al. also reports that low circulating adiponectin levels are associated with MM risk in overweight and obese individuals [76]. Long-term and short-term consequences of dysfunctional neighboring BM cells and elevated MAT on lineage selection, immune function, bone strength, and systemic endocrine signaling remain incompletely defined, but likely are a net negative for human health.

Effects of adiponectin on bone and MM are controversial and model dependent. Adiponectin is derived from adipocytes, circulates systemically in plasma, and is responsible for regulating blood glucose and the oxidation of fatty acids. Adiponectin has also been shown to inhibit growth and proliferation of cancer cells and prevents angiogenesis at tumor sites [77, 78]. Additionally, circulating levels of adiponectin are inversely proportional to fat mass [79], and adiponectin has been shown to inhibit proliferation of MM through an increase in cell death via activation of the protein kinase A/AMP-activated protein kinase pathways [74]. Inhibition of MM cells via adiponectin occurs through several signaling pathways, including signal transducer and signal activator 3 (STAT3), mitogen-activated protein kinase (MAPK), cyclic AMP-dependent protein kinase A (PKA), β -catenin and phosphatidylinositol 3-kinase (PI3K/AKT). This results in the anti-proliferative and anti-tumorigenic effects of adiponectin observed in MM, breast, prostate, colon and liver cancers [74, 78]. Overweight and obese individuals typically present with low levels of adiponectin and are at a greater risk of developing MM when compared to individuals of healthy weight [80, 81]. Mechanistically, deficiency in adiponectin hinders the biological actions of several signaling pathways that are essential to prevent growth, proliferation, migration, and drug resistance of MM cells [81], and adiponectin can alter bone turnover for net bone anabolic or

catabolic effects [82, 83]. Hence the net effects of adiponectin on myeloma-induced bone disease and tumor burden remain controversial. Decreasing MAT may result in stronger bones, while strengthening bones may result in decreased MAT through feedback systems that may prove to be beneficial when targeting the microenvironment for MM and other tumor cells within the BM. More on the specifics of MAT-MM interactions can be found in our recent publication on this topic [84].

Despite recent advances in our understanding of the MM-adipocyte relationship, there remains a great need for research into the interactions between adipocytes and myeloma cells to reveal novel therapeutic targets. Specifically, more investigation into marrow adipocyte support of myelomagenesis, myeloma cell proliferation, immune evasion, drug resistance, and distant spreading will enable the development of better therapies and prevention strategies. In sum, a cohesive story explaining the net effects of BM adiposity on MM is greatly needed.

Bone Marrow-Derived MSCs (BM-MSCs)

BM-MSCs can support bone-metastatic cancers in a variety of manners. In MM, BM-MSCs support tumor colonization and growth within the bone marrow, and this function has been shown to depend on the donor (myeloma patient or healthy control) and DKK-1 expression [85]. Specifically, BM-MSCs have been shown to support tumor growth, metastasis, chemoresistance, survival, and evasion of the immune system [86]. Interestingly, compared to normal-donor MSCs (ND-MSCs), MSCs from myeloma patients (MM-MSCs) are significantly different in terms of their proliferative rate, function, mRNA and microRNA expression, composition and effects of their secreted exosomes [87], tumor support, and other properties [54, 86]. Moreover, we recently observed that the osteogenic potential of MM-MSCs is decreased compared to ND-MSCs, and that this is due, in part, to altered microRNA expression [54]. Importantly, whereas MM-MSC-derived exosomes promoted MM tumor growth, normal BM-MSC-derived exosomes inhibited the growth of MM cells in a recent study by Ghobrial et. al. [87]. This work also demonstrated, *in vivo* and *in vitro*, microRNA-containing exosome transfer from BM-MSCs to MM cells, highlighting a new mechanism through which BM-MSCs contribute to MM disease progression [87]. Since BM-MSCs can differentiate into BM adipocytes or osteoblasts, inducing or changing the differentiation of these cells may be a new therapeutic target in MM.

Perivascular cells

The perivascular niche is rich in blood vessels that are composed of endothelial cells and mural cells (pericytes and smooth muscle cells), as described above. As with HSCs, the perivascular niche plays a large role in directing MM cell homing to, engraftment in, and dissemination from the BM. Indeed, MM cells preferentially engraft in the metaphysis of the bone due in part to its rich vascularization [88]. Osteolytic bone metastases often cause new, abnormal blood vessels to sprout from the periosteum, which replace the healthy vessels from the medullary artery from the diaphyseal shaft. These new cancer-associated vessels are irregular in diameter with a tortuous, disorganized architecture, and increase with increasing tumor growth [88]. The amount of vascularization within a myeloma tumor in the BM directly correlates with tumor burden, and the vascular component of the tumor plays an

important role in supporting MM cell growth and chemoresistance [89]. Research from Ghobrial et al. demonstrates that endothelial progenitor cells are mobilized to the blood in early stages of MM, and are recruited to MM cell-colonized BM niches where they enhance proliferation and cell cycle progression in smoldering-like MM clones. In sum, the perivascular niche represents another location where BM cells support long term dormancy of MM cells, MM growth, and disease progression [90].

4. Targeting BM elements to control disease (Bone/Niches/Adipose)

Therapies Targeting Bone Cells

Numerous niche-targeted therapeutics are in development or currently used, and numerous other therapeutics, such as the immunomodulatory drugs (IMiDs, e.g., thalidomide, lenalidomide, palmaridomide) have direct effects on niche cells as well as MM cells (Table 1) [91–101]. A number of bone targeted anti-resorptive therapies (bisphosphonates) are used in MM and anabolic (bone-forming) agents are currently in clinical trials for MM. The aim of these bone targeted therapies is to address MM-induced loss of bone structure and integrity. Bisphosphonates are the major anti-osteoclast therapy and have been successful at slowing bone resorption. Importantly, they also reduce the incidence of SREs [2]. The RANKL-neutralizing antibody denosumab is currently being explored in a Phase III clinical trial (ClinicalTrials.gov Identifier: NCT01345019); survival results in MM patients at this point are however too preliminary to analyze for reliable conclusions [102]. The cathepsin K inhibitor, odanacatib, is also currently in clinical trials for breast cancer bone metastasis; odanacatib has been shown to reduce bone resorption markers after 4 weeks of treatment [103] and therefore has potential for translation to MM patients. Bisphosphonates prevent further bone loss in MM patients with improved outcomes in regards to fractures, and likely other anti-resorptive agents will demonstrate this ability as they become tested in MM as well. However, MM patients continue to fracture despite bisphosphonate therapy, as lost bone is not restored by these agents. As a result, emerging therapies which have the potential to rebuild bone have great promise to improve outcomes.

Anabolic agents which promote bone formation include activin inhibitors, anti-Dkk1, and more recently anti-sclerostin antibodies. These agents increase osteoblast differentiation and activity via promotion of either smad or Wnt signaling pathways, further they also target the Wnt and smad signaling pathways, which have been implicated in the development of MM bone disease. The activin-A inhibitor sotatercept (ACE-011), a soluble activin receptor type 2A IgG-Fc fusion protein that inhibits activin-A and downstream smad signaling, is in the recruiting stage of a phase I trial (ClinicalTrials.gov Identifier: NCT01562405). This drug is being tested in combination with lenalidomide or dexamethasone in MM patients, based on preclinical and clinical data supporting the rationale for the use of activin-A antagonists in MM [104–106]. A recently completed phase II trial in bisphosphonate-naïve MM patients with osteolytic lesions (ClinicalTrials.gov Identifier NCT00747123) showed increased bone-specific alkaline phosphatase and decreased bone resorption marker C-terminal telopeptide (CTX) when ACE-011 was added to a melphalan and prednisone regimen [107]. Along a similar vein, anti-Dkk1 antibodies neutralize Dkk1 antagonism of Wnt signaling and drive osteoblast differentiation via promotion of Wnt signaling. Following successful pre-clinical

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studies in preventing the development of osteolytic lesions in myeloma [107], the Novartis Pharmaceuticals anti-DKK1 antibody BHQ880 proved to be well tolerated in a Phase IB trial [108] and has been tested in a Phase II high-risk smoldering MM clinical trial (ClinicalTrials.gov Identifier NCT01302886) with results pending. HealthCare Pharmaceuticals, Inc. DKK1 neutralizing antibody (DKN-01) also completed testing in a phase 1/2 clinical trial (ClinicalTrials.gov Identifier: NCT01711671) and results are forthcoming. The proteasome inhibitors bortezomib and carfilzomib have also been shown to display bone anabolic properties, increase osteogenic differentiation *in vitro*, and increase bone parameters such as bone volume per total volume *in vivo* [91, 109]. Therefore, the bone-building effects of these proteasome inhibitors may also combat myeloma-induced bone disease, and could decrease tumor proliferation through a feedback mechanism resulting from increased osteoblast numbers.

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Another Wnt promoting agent in clinical development is an anti-sclerostin antibody. Anti-sclerostin antibodies are in completion of phase III trials for rebuilding bone mass in osteoporosis patients with exciting outcomes (ClinicalTrials.gov Identifier NCT01631214). Anti-sclerostin treatment holds excellent clinical promise not only due to its potent anabolic effects, based on *in vivo* data, but also due to its high specificity for a protein which is primarily produced by osteocytes and therefore has few off-target effects. To date, therapies which target the osteocyte specifically are lacking, in part due to the elusive role osteocytes play in MM bone disease. As it appears that the endosteal niche is responsible for retaining quiescent MM cells, augmenting the endosteal niche with therapies such as anti-sclerostin antibodies, which increase osteoblast numbers and total bone volume, may induce tumor quiescence and extend patient lifetimes while decreasing MM bone disease. However, the negative consequence of this action may be that more MM cells are retained in their chemoresistant state. Thus, intelligent drug combinations and schedules should be designed to optimize the use of the bone-targeted treatment in MM.

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Although bone-targeted therapies are aimed at preventing MM-induced bone loss, interesting data have revealed a role for them in regard to tumor growth. This is of particular interest for agents which stimulate Wnt signaling, a well-established pro-tumorigenic pathway. Anti-resorptive agents such as bisphosphonates and RANK-targeted treatments (OPG and denosumab) impact tumor growth in bone, evidenced robustly in pre-clinical MM models [110, 111] with recent data implicating bone resorption in the activation of tumor initiating cells [53]. Therapies that were not originally targeted at the perivascular niche may also inhibit angiogenesis and therefore tumor growth; for example, the inhibition of osteoclasts using bisphosphonates reduces angiogenesis and tumor burden in MM [110]. Importantly, recent clinical analyses highlight a reduced recurrence of skeletal metastases in breast cancer patients on bisphosphonates [112] while denosumab treatment increased metastasis free survival in men with prostate cancer [113]. Hence, anti-resorptive agents provide dual action treatment in cancer, preventing further bone loss and suppressing tumor growth. Whether or not agents which promote bone formation will impact tumor growth requires further investigation, particularly in the clinical setting.

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In pre-clinical studies, Activin A inhibition had only minimal impact on bone marrow tumor growth, but it did improve time to morbidity in MM bearing mice [106]. Further, in mice

bearing breast cancer bone metastases, bone tumor growth was inhibited with Activin A inhibition. Anti-Dkk1 treatment has also been associated with suppression of tumor growth in pre-clinical models of MM [114–116], other studies show no impact on tumor growth [117]. These pre-clinical tumor results are awaiting validation through clinical trials, and although ambiguous, these data suggest that targeting osteoblast activity may indirectly suppress tumor growth. It is therefore important that studies investigating these agents in pre-clinical models of MM thoroughly investigate tumor outcomes to determine if they may have dual actions. As we learn more about the advantages of targeting the BM and the potential for building bone back in order to combat the tumor and bone disease, we anticipate that emerging microenvironmentally-targeted therapeutics may also interfere with MM-supportive properties of the niche.

Therapies Targeting Adipocytes

It is currently neither realistic nor pragmatic to target one BM cellular component in the management of MM without considering the influences of such a treatment on other cells of the BM. With a more concerted research emphasis placed on co-targeting cell types, such as osteoblast/adipogenic lineage cells, their common progenitors, or their lineage switch transcription factors, more rapid development of more efficacious therapies could result. Wnt signaling is known to push mesenchymal stromal cells down the osteogenic lineage and inhibit their differentiation down the adipogenic lineage. On this basis, targeting this pathway has the potential to strike as a two-pronged attack, both increasing bone and decreasing adipose within the marrow cavity. Because Wnt inhibitors such as DKK1 and sclerostin are elevated in MM, inhibiting them with DKK1 or anti-sclerostin antibodies may not only increase bone parameters, but also decrease BMAT in patients; this remains to be seen [8, 69].

Since modern treatments for MM may exacerbate patients' bone disease and increase their risk for obesity, targeting MAT may expand as a field of interest for pharmaceutical companies and researchers alike. For example, endocrine, metabolic, nutritional, and body composition abnormalities are common in advanced intensively-treated (transplanted) MM patients; this further complicates the interpretation of the roles of MAT in disease progression and the evaluation of the best treatment approaches [81]. Specifically, intensively treated patients had a high prevalence of endocrine dysfunction (hypothyroidism (9%), hypogonadism (65% males), and elevated prolactin (19%)). Also, biochemical markers were consistent with post-menopausal status in all females and infertility was high in males and Vitamin D, B12, and folate deficiencies, as well as "sarcopenic-obesity", were observed in many MM patients [81]. In order to combat the potential that elevated MAT impacts tumor growth in MM, a number of approaches currently exist in the clinic. Weight loss via diet and exercise has been shown to decrease elevated MAT resulting from high fat diet or PPAR γ agonists [25, 118]. Elevated MAT can also be treated with the anti-diabetic drug metformin, which is able to significantly decrease MAT in a diet-induced obesity mouse model (unpublished data). The potentially correctable endocrine, metabolic, and nutritional abnormalities prevalent in heavily-treated patients with stable MM should be addressed, potentially with dietary supplements, bisphosphonates (to increase bone mass), and other interventions (weight training/weight loss) in order to optimize long-term patient

survival and quality of life. With this in mind, multi-system screening would be beneficial for patient-centric care. Further studies are warranted to assess endocrine, metabolic, nutritional, and body composition characteristics, for MM patients spanning from MGUS to relapse/refractory. As more is understood regarding the cellular biology of the BM adipocyte, it is likely that new treatments targeting MAT will be developed.

5. Conclusions

Bone resident cells, including osteoclasts, osteoblasts, osteocytes, BM adipocytes, and MSCs, exist in a complex microenvironment in the BM niche. These cells interact closely, are influenced by many factors, and should all be taken into consideration when developing new approaches for MM therapy. Ideally, a maximally effective therapy that targets the BM niche should have effects on multiple BM cell types, to both inhibit MM growth and repair osteolytic bone disease. By understanding the interactions of the cells in the BM in a healthy state more deeply, researchers will be better positioned to predict how MM disrupts or interacts with the normal BM and therefore design therapies to target this interaction. Grasping the roles of BM cells in stimulating MM progression will also help identify novel BM-derived biomarkers or indicators, rather than tumor cell derived biomarkers, which could better predict which patients may progress into MM and which may remain stable for years. By targeting BM cells rather than MM tumor cells, we may be more successful at overcoming the issues inherent in treating a heterogeneous, clonal, and constantly-evolving population of tumor cells. Lastly, we propose that innovative *in vivo* and *in vitro* systems for the study of MM and bone should be designed, and that more research should be directed at understanding the BM microenvironment in order to aid the development of more effective therapeutics.

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depending on the activity of the surface they engage with. Osteoblasts are inhibited, osteoclasts are supported, and effects on adipocytes are largely unknown. Hematopoiesis is also disrupted as the tumor grows, and the immune system's normal anti-tumor function is inhibited.

Figure 2. Osteoclast-Myeloma Interactions

Myeloma cells induce a vicious cycle in the bone marrow where osteoclasts are activated by myeloma cells through molecules such as RANKL, MIP1 α , TNF- α , IL-3 and IL-6. In turn, osteoclasts support MM cells through secretion of molecules such as IL-6, OPN, and TGF- β and release of growth factors, collagens, and other proteins stored in the bone matrix. By resorbing matrix, osteoclasts may also release dormant MM cells, transforming them into activated tumor cells which lead to micrometastases.

Figure 3. Osteoblast/Osteocyte- Myeloma Interactions

Myeloma cells inhibit osteoblastogenesis and osteoblastic activity through secretion of molecules such as Dkk-1, SFRP-2/3, and TNF- α . MM cells also induce apoptosis in osteocytes, and increase their secretion of sclerostin, through Notch signaling. Bone lining cells of the endosteal niche are able to induce dormancy and potentially apoptosis in MM tumor cells.

Figure 4. Bone Marrow Adipocyte-Myeloma Interactions

Correlative and preliminary data suggest that bone marrow adipose tissue supports myeloma tumor cells through various mechanisms. Excessive bone marrow adiposity has been linked to decreased osteoblast and immune cell function, which may indirectly accelerate MM cell proliferation. BM adipocytes also directly support tumor growth via secretion of adipokines (leptin and resistin) and potentially lipids as a high-caloric energy source for tumor cells. Obesity and elevated BMAT may also lead to hypertrophy or apoptosis in BM adipocytes, which could cause local inflammation and may accelerate tumor growth through enhanced COX-2 or CCL-2 signaling in tumor cells. Influences of MM cells on BMAT are essentially unknown and under investigation.

Table 1

Niche targeted therapeutics

Therapeutic	Niche Target	Tumor/Survival Effects	Phase
Bisphosphonates	Osteoclasts (109)	Anti-tumor effects Increased relapse-free survival Improved overall survival (93,101,102)	Clinical Use
Denosumab	Osteoclasts	No current evidence for anti-tumor effects (102)	Phase III clinical trials
Odanacatib	Osteoclasts	Unknown in MM	Phase II clinical trials in metastatic BC
Activin-A inhibitors	Osteoclasts Osteoblasts (104,106)	Unknown	Phase IIb clinical trials
Anti-Dkk1	Osteoblasts (115–117)	Pre-clinical evidence for reduced tumor burden (114)	Phase II clinical trials (108)
Anti-Sclerostin	Osteoblasts	Unknown, preclinical data only	Not commenced
Bortezomib	Tumor cells Osteoblasts (91) Osteoclasts (94)	Reduced tumor Improved Survival	Clinical Use
Carfilzomib	Tumor cells Osteoblasts Osteoclasts (95)	Reduced tumor Improved survival (95)	Clinical Use
Ixazomib	Tumor cells Osteoblasts Osteoclasts	Reduced tumor Improved Progression free survival in combination therapy (97)	Clinical Use
Pomalidomide	Osteoclast Precursors (Decreased osteoclastogenesis) (98)	Extension of progression-free survival and overall survival when used with dexamethasone (99)	Clinical Use
Thalidomide and Lenalidomide	Decreased osteoclastogenesis Decreased osteoblastogenesis * (100) (disputing prior data showing no effects on osteoblasts)(101)		Clinical Use

* Note, this is a detrimental side-effect of anti-myeloma agent on bone.