Bone Disease in Multiple Myeloma

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Abstract

Bone involvement represented by osteolytic bone disease (OBD) or osteopenia is one of the pathognomonic and defining characteristics of multiple myeloma (MM). Nearly 90 % of patients with MM develop osteolytic bone lesions, frequently complicated by skeletal-related events (SRE) such as severe bone pain, pathological fractures, vertebral collapse, hypercalcemia, and spinal cord compression. All of these not only result in a negative impact on quality of life but also adversely impact overall survival. OBD is a consequence of increased osteoclast (OC) activation along with osteoblast (OB) inhibition, resulting in altered bone remodeling. OC number and activity are increased in MM via cytokine deregulation within the bone marrow (BM) milieu, whereas negative regulators of OB differentiation suppress bone formation. Inhibition of osteolysis and stimulation of OB differentiation leads to reduced tumor growth in vivo. Therefore, novel agents targeting OBD are promising therapeutic strategies not only for the treatment of MM OBD but also for the treatment of MM. Several novel agents in addition to bisphosphonates are currently under investigation for their positive effect on bone remodeling via OC inhibition or OB stimulation. Future studies will look to combine or sequence all of these agents with the goal of not only alleviating morbidity from MM OBD but also capitalizing on the resultant antitumor activity.

Keywords

Multiple myeloma · Bone disease · Therapies

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1 Introduction

The past two decades have seen remarkable advances in our understanding of the biology of multiple myeloma (MM) and in the introduction of novel therapies. Novel treatments including thalidomide [1], lenalidomide [2], and the proteasome inhibitor bortezomib [3] have led to significant improvements in 5-year relative overall survival, from nearly 28.8 % in the early 1990s to 34.7 % in the previous decade [4]. Although MM remains incurable, MM patients are living longer, and the focus is centered on maximizing quality of life for patients with MM.

Bone involvement represented by osteolytic bone disease (OBD) or osteopenia is one of the pathognomonic and defining characteristic of MM [5]. Although the ratio of patients presenting with bone involvement is variable, nearly 90 % of patients with MM develop osteolytic bone lesions, frequently complicated by skeletal-related events (SRE) such as severe bone pain, pathological fractures, vertebral collapse, hypercalcemia, and spinal cord compression, resulting in a need for radiation or open reduction internal fixation (ORIF) [6–10]. Importantly, 40–50 % of MM patients develop pathologic fractures, and it increases the risk of death by more than 20 % compared with patients without fractures [8, 11]. These data indicate how OBD negatively impact both patients' quality of life and survival, and highlight the importance of focusing on treatment strategies to alleviate OBD in MM.

OBD results from the disruption of the delicate balance between osteoclasts (OCs), osteocytes, osteoblasts (OBs), and bone marrow stromal cells (BMSCs) activity. MM cells stimulate OC function and inhibit OB differentiation, resulting in bone resorption and consequent OBD. The abnormal bone marrow (BM) microenvironment in OBD provides a permissive niche that enables MM cell growth [9, 12–14]. Consequently, several novel agents and combinations are aimed at restoring bone homeostasis by targeting either OC or/and OB activity. In fact, inhibition of osteolysis and stimulation of OB differentiation leads to reduced tumor growth in vivo [13, 15]. Therefore, novel agents targeting OBD are also promising therapeutic strategies for the treatment of MM. Here, we discuss the pathogenesis of OBD and focus on advances in our understanding of its biology and therapeutic implications.

2 The Biology of Bone Metabolism

Under normal physiologic states, osteocytes, OCs and OBs result in balanced bone resorption and formation maintaining normal homeostasis. In adult bone, 90–95 % of all bone cells are represented by osteocytes while OCs and OBs are less than 10 % [16]. Osteocytes act as main regulators of bone homeostasis for OCs, considered bone resorption cells, and OBs considered bone formation cells. Osteocyte viability and function is regulated by mechanical loading, several cytokines

includes well as glucocorticoids [16–18]. Osteocytes secrete several cytokines which regulate the activity of both OCs and OBs such as sclerostin, dickkopf-1 (Dkk-1), the receptor activator of nuclear factor-kappa B ligand (RANKL), and osteoprotegerin (OPG) [16]. The receptor activator of nuclear factor-kappa B (RANK), its ligand RANKL, and OPG, the decoy receptor of RANKL, play a pivotal role as central regulators of OC function. RANK-RANKL signaling activates a variety of downstream signaling pathways required for OC development. It plays a significant role in stimulating OC differentiation and maturation. Interestingly, apoptotic osteocytes release apoptotic bodies expressing RANKL to stimulate OC differentiation [19]. These data suggest that osteocytes are able to recruit OCs to sites of remodeling. Osteocytes also regulate OB differentiation via sclerostin and Dkk-1 which block canonical Wnt signaling by binding to low-density lipoprotein receptor-related protein (LRP) 5 and 6 (Wnt receptors) on the surface of OBs [16]. OBs and BMSCs also express OPG and RANKL, and regulate OC differentiation. Because OPG is a Wnt canonical signaling target [20], osteocyte also regulates OC differentiation via regulation of Wnt signaling activity in OBs. On the other hand, OCs express semaphorin 4D (Sema4D) and inhibit OB differentiation [21]. These processes are well balanced in healthy bones to maintain the bones quality and mass (Fig. 1).

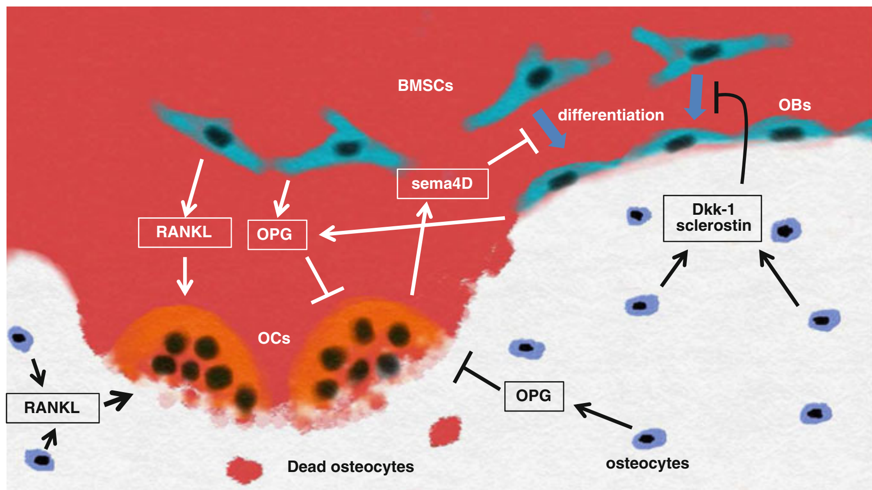

Fig. 1 Healthy Bone metabolism. Osteocytes regulate OC (osteoclast) and OB (osteoblast) differentiation. OBs also regulate OC differentiation. On the other hand, OCs can inhibit OB differentiation. These mechanisms are well balanced in healthy bones to keep the bones quality and mass

3 MM Bone Disease

In MM, the osteocyte-OC-OB axis is disrupted, stimulating bone resorption and inhibiting new bone formation with resultant development of pathognomonic osteolytic lesions (Fig. 2).

3.1 Osteoclasts in Myeloma Bone Disease

The pathogenesis of OBD in MM is primarily associated with generalized OC activation. BM biopsies from MM patients show a correlation between tumor burden, OC numbers, and resorptive surface [22, 23]. Furthermore, OC activity has positive correlation with disease activity [24, 25]. The main cytokines involved in OC differentiation and activity in MM OBD are RANKL/OPG, decoy receptor 3 (DcR3), CCL3 (also known as macrophage inflammatory protein (MIP)-1α), MIP-1β, tumour necrosis factor-alpha (TNFα), interleukin (IL)-3, IL-6, IL-11,

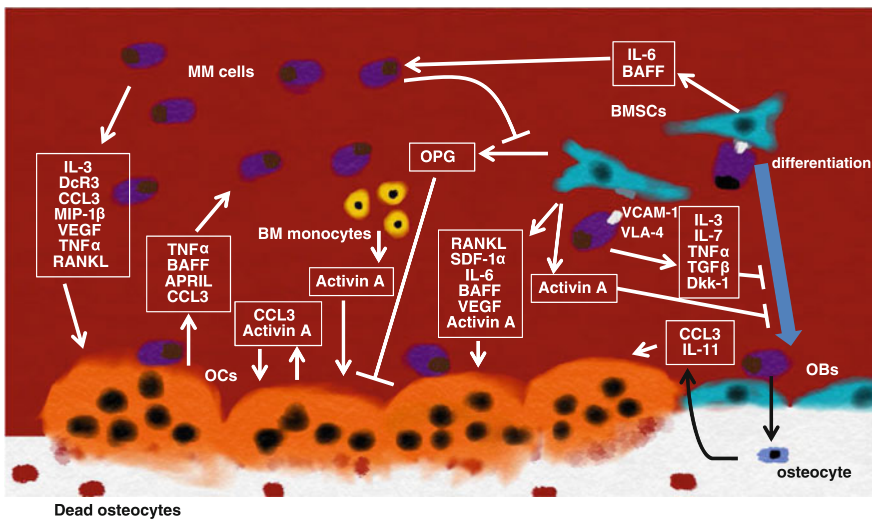

Fig. 2 Myeloma Bone Disease. MM cells produce IL-3, DcR3, CCL3, MIP-1β, VEGF, TNFα, and RANKL. MM cells also adhere to BMSCs via VLA-4 and VCAM-1 interaction, and lead to the secretion of RANKL, SDF-1a, IL-6, BAFF, VEGF, and activin A. Moreover, MM cells stimulate CCL3 and IL-11 expression in osteocytes. OCs secrete CCL3 and activin A by MM cells stimulation. These cytokines stimulate OC differentiation and activity. MM cells also inhibit OPG expression in BMSCs and OBs resulting in stimulation of OC differentiation. On the other hand, MM cells produce IL-3, IL-7, TNFα, TGFβ, and Dkk-1. MM cells also stimulate activin A expression in BMSCs. These cytokines inhibit OB differentiation. Stimulated OCs destroy bone matrix, and release several tumor growth factor from bone. Moreover, OCs and BMSCs express several cytokines. These cytokines mediate MM cell survival and proliferation

Stromal derived factor-1 alpha (SDF-1a), B-cell activating factor (BAFF), activin A, and VEGF.

MM cells stimulate OC differentiation by producing IL-3 [26], DcR3 [27, 28], CCL3, MIP-1β [29–31], VEGF [32], TNFα, [33, 34] and RANKL [35–38]. MM cells also adhere to BMSCs via very late antigen (VLA)-4 and vascular cell adhesion molecule (VCAM)-1 interaction leading to the secretion of cytokines including RANKL, SDF-1a, IL-6, BAFF, VEGF, and activin A which in turn positively affect OC differentiation and activation [9, 14, 32, 39–43]. MM cells stimulate not only RANKL expression, but also inhibit OPG expression, leading to an increase in RANKL/OPG ratio in BMSCs and OBs which in turn strongly stimulate OC differentiation [24, 44]. In addition to BMSCs and OBs, MM cells also stimulate CCL3 and pro-osteoclastogenic cytokine, IL-11 in osteocytes [45]. Moreover, OCs secrete CCL3 and activin A, and stimulate OC differentiation and activation by themselves [9, 31]. BM macrophages stimulated by IL-3 also secrete activin A [46]. All these cytokines stimulate OC differentiation and activity, and contribute to the development of MM OBD.

3.1.1 CCL3

CCL3 is a pro-inflammatory cytokine belonging to the CC chemokine subfamily. High CCL3 levels were found in MM patients' BM serum and it correlates with OBD and survival [30]. Interestingly, fibroblast growth factor receptor 3 (FGFR3) overexpression in MM with t(4,14) results in upregulation of CCL3 expression [47]. CCL3 modulates OC differentiation by binding to G-protein coupled receptors, CCR1 and CCR5, and activating ERK and AKT signaling pathways. CCL3 has the ability to stimulate OC differentiation not only from monocytes but also from immature dendritic cells by transdifferentiation [48]. In the tumor niche, MM cells and OCs are the main source for CCL3 that promotes MM cell migration and survival, along with stimulation of osteoclastogenesis [49, 50]. Vallet et al. also showed that CCL3 reduces bone formation by inhibiting OB function by ERK activation and followed by down regulation of the osteogenic transcription factor, osterix [31]. Importantly, a small molecule CCR1 antagonist inhibits CCL3-induced osteoclastogenesis and OC support of MM cells [51].

3.1.2 RANKL to OPG Ratio

Many of the cytokines which stimulate OC differentiation and activity act through RANKL and OPG. Increase of the RANKL to OPG ratio results in bone loss in several cancers and inflammatory diseases including rheumatoid arthritis [52–54]. In MM patients, BM plasma levels of RANKL are increased, whereas OPG expression is decreased compared with normal volunteers and patients with MGUS [35]. Importantly, low levels of OPG in serum correlate with advanced OBD in MM [55]. The relevance of the RANKL/OPG pathway in mediating OC differentiation and activation in MM has been further confirmed in several murine models of MM OBD. Treatment with OPG or OPG-like molecules prevented both bone destruction and MM growth in vivo [36, 56]. Interestingly, specific anti-MM strategies such as thalidomide and autologous BM transplantation improved OBD

by normalizing the RANKL to OPG ratio [57, 58]. Therefore, the RANKL-OPG axis is one of the important targets in the development of novel therapeutic strategies for MM bone disease.

3.2 Bone Marrow Stromal Cells and Osteoblasts in Myeloma Bone Disease

Besides OCs, BMSCs and OBs derived from BMSCs, play an important role in the development of OBD in the presence of MM cells. MM cells stimulate OC differentiation directly by secreting OC-activating factors (OAFs) and, indirectly, by stimulating OAFs secretion such as RANKL, Activin A and VEGF in BMSCs and OBs [14, 35, 36, 59, 60]. Adhesion of MM to BMSCs leads to RANKL and VEGF secretion by BMSCs via p38 MAPK activation [59, 60]. Moreover, the sequestosome 1, p62 is an upstream regulator of p38 MAPK and NF-κB signaling pathway, activated in BMSCs by MM cell adhesion. Inhibition of p62 in BMSCs represses OC differentiation and MM cell proliferation [61]. These data suggest that p62 is a novel promising target in MM OBD. Adhesion of MM to BMSCs and immature OBs also leads to IL-6 secretion via NF-κB signaling [42, 43, 62] and X-box-binding protein 1 (XBP1) signaling [63] pathway. IL-6 stimulates MM cell proliferation and inhibition of MM plasma cell apoptosis [64] in addition to OC differentiation. Moreover, adhesion of MM cells also stimulates BAFF expression in BMSCs via NF-κB signaling [41]. BAFF is a MM cell survival factor and it rescues MM cells from apoptosis induced by IL-6 deprivation and dexamethasone via activation of NF-κB, phosphatidylinosiol-3 (PI-3) kinase/AKT, and MAPK pathways in MM cells and induction of a strong upregulation of Mcl-1 and Bcl-2 antiapoptotic proteins [65, 66]. Secreted IL-6 and BAFF also stimulates the serine/threonine kinase Pim-2 expression in MM cells via activation of NF-κB and JAK2/STAT3 pathway, resulting in MM cell survival [67]. Furthermore, MM cells stimulate activin A expression in BMSCs via Jun N-terminal kinase-dependent (JNK) activation [9]. Importantly, high activin A levels in MM patients are associated with advanced bone disease and advanced features of MM [68]. Secreted Activin A inhibits OB differentiation in addition to the growth stimulatory effects on OCs. MM cells also stimulate Pim-2 expression in BMSCs/OBs by IL-3, IL-7, TNF-a, TGF-β and activin A secretion, and inhibit OB differentiation [69].

3.2.1 Wnt Canonical Signaling in BMSCs and OBs

Wnt canonical signaling plays an important role in OB differentiation. Some Wnt proteins bind to both Frizzled and LRP 5 and 6, and activate Wnt canonical signaling. Activated Wnt signaling induces nuclear translocation of β-catenin protein resulting in stimulation of OB differentiation by activation of major OB transcription factors [70]. Wnt antagonists, such as Dkk-1, sclerostin and secreted frizzled related proteins (sFRPs) inhibit Wnt canonical signaling activity by blocking Wnt proteins binding to Wnt receptors. Thus, these Wnt antagonists act as negative regulators for OB differentiation. In MM OBD, OB differentiation is

strongly inhibited. MM cells secrete several Wnt antagonists such as Dkk-1 [71], sFRP-2 [72], sFRP-3 [73] and inhibit Wnt canonical signaling. High Dkk-1 levels have been detected in MM patients' serum and have been correlated with MM bone lesions [71]. Also high circulating levels of sclerostin, encoded by the SOST gene, have been found in newly diagnosed MM patients, and correlates with MM disease stage and fractures [74]. There is a report that MM cells produce sclerostin [75], however, we and others [76] could detect very little sclerostin or SOST mRNA expression in MM cell lines. The source and role of sclerostin in MM OBD therefore remains to be defined. Importantly, Wnt antagonists inhibit OPG expression as OPG is a target of Wnt canonical signaling [20], and increase the RANKL to OPG ratio. They are responsible not only for suppression of OB differentiation and activity but also for stimulation of OC differentiation and activity in MM OBD.

3.3 Osteocytes in Myeloma Bone Disease

Osteocytes act as main regulators of bone homeostasis in healthy bone [16]. A recent study showed that MM patients have a significantly lower number of viable osteocytes than healthy controls, and that osteocyte death correlates with the presence of bone lesions [45]. Besides a lower number of viable osteocytes has been observed in the MM patients, no significant difference in the expression of sclerostin, an osteocyte marker, in biopsies of MM patients bone and healthy controls osteocyte was observed [45]. On the other hand, higher circulating levels of sclerostin have been found in newly diagnosed MM patients as mentioned before [74]. These data suggest that there might be other alternate sources of sclerostin in addition to osteocytes in MM. Moreover, MM cells stimulate osteoclastogenic cytokines, CCL3 and IL-11 expression in pre-osteocytes leading to increased OC differentiation [45]. Further investigations regarding the role of osteocytes in MM OBD are underway.

4 Treatment of Myeloma—Related Osteolytic Bone Disease

Current treatment strategies in MM have led to significant improvements in 5-year relative overall survival, but patients continue to relapse, and no definitive cure has been as yet achieved. Given the improved survival of MM patients, treatment of OBD has taken on a new relevance as the focus is now largely on quality of life. Until recently, therapeutic options for MMOBD-included bisphosphonates, radiotherapy, and surgery. These therapies are aimed at reducing the development of new osteolytic lesions and preventing SREs such as bone pain, pathological fractures, vertebral collapse, hypercalcemia, and spinal cord compression. Interestingly, several studies using novel bone-targeted agents suggest that restoring bone

Table 1 Bone-Directed Therapies for Multiple Myeloma

Drug	Role	Target	Clinical Development
Bisphosphonates	FPPS inhibition (in OC)	OCs	Approved
Pamidronate Zoledronic acid etc.	ERK activation (in OB and osteocyte)	OBs and osteocytes	
RANKL antagonist			
Denosumab	Neutralizing antibody for RANKL	OCs	Phase III clinical trials
OPG agonist			
AMGN-0007	Recombinant OPG	OCs	Phase I clinical trials
CCR1 inhibitor			
MLN3897	small-molecule CCL3 receptor antagonist	OCs	Preclinical studies
Dkk-1 antagonist			
BHQ880	Neutralizing antibody for Dkk-1	OBs	Phase II clinical trials
Sclerostin antagonist			
romosozumab blosozumab	Neutralizing antibody for sclerostin	OBs	Preclinical studies
Proteasome inhibitor			
bortezomib carfilzomib	26s proteasome inhibition 20s proteasome inhibition	Anti-MM and OCs OB stimulation	Approved
Btk inhibitor	Btk inhibition	OCs	Preclinical studies
CC-292 PCI-32765 LFM-A13			
Pim inhibitor	Pim inhibition	Anti-MM OB stimulation	Preclinical studies

homeostasis may lead to tumor growth inhibition. These promising preclinical results have set the stage for clinical evaluation of novel strategies targeting MM via restoring bone homeostasis. Table 1 provides a list of bone-directed agents, their roles, targets, and stage of clinical development.

4.1 Bisphosphonates

Bisphosphonates represent the standard of care for MM OBD. Nitrogen-containing bisphosphonates such as pamidronate (PAM) or zoledronic acid (ZA), more potent than PAM, reduce osteoclast activity through inhibiting farnesyl pyrophosphate synthase (FPPS) [77]. Bisphosphonates prevent OB and osteocyte apoptosis with a different mechanism from the effect on OCs [78–80]. Bisphosphonates induce ERK activation without nuclear accumulation in OBs and osteocytes. Activated ERK stimulates p90RSK and induces phosphorylation of the cytoplasmic substrates, BAD and C/EBP, which are required for OB and osteocyte survival [81].

Bisphosphonates have a well-established role in the treatment of osteoporosis [82, 83] and metastatic bone involvement from solid tumors [84–86]. In MM, treatment with bisphosphonate significantly reduces pain related to OBD and prevents SREs. Monthly infusion of PAM reduces bone pain and SREs compared with placebo [87]. PAM also significantly improved quality of life, with decreases in pain scores seen within a month. Moreover, Major et al. reported that ZA was superior to PAM for the treatment of hypercalcemia of malignancy including MM [88] although Rosen et al. reported the efficacy of ZA in preventing SREs in MM was comparable to that of PAM [84].

In addition to their role in OBD, bisphosphonates may also have an antitumor effect. The Austrian Breast and Colorectal Cancer Study Group 12 (ABCSG-12) trial showed that the administration of zoledronic acid every 6 months for 3 years reduced the risk of disease recurrence in estrogen-receptor—positive breast cancer patients [89] although no improvement was seen in the rate of disease-free survival in another study [90]. In MM, The MRC Myeloma IX trial compared ZA and oral clodronate in newly diagnosed patients and found that ZA reduced mortality by 16 % and increased median overall survival from 44.5 to 50.0 months ($P = 0.04$) [91]

4.1.1 Osteonecrosis of the Jaw

Bisphosphonate-related osteonecrosis of the jaw (BRONJ) is one of the most serious complications of bisphosphonates [92, 93]. BRONJ is traditionally defined as exposed, necrotic bone in the jaw that does not heal after 8 weeks and is generally painful. Histologically, several tissue alterations such as honeycombed-like necrotic bone with residual vital bone, inflammatory cellular elements, and hypernucleated osteoclasts are observed in BRONJ [94–96]. ZA is associated with the highest risk of BRONJ, attributed to its increased potency, and earlier studies suggested an incidence of 4–11 %, correlating with duration of exposure [97, 98]. In the MRC Myeloma IX trial, the cumulative incidence of BRONJ was 3–4 % at a median follow-up of 3.7 years [99]. It is clear that trauma, infection, and reduced vascularity including dental extractions play important roles, however, the exact etiopathogenetic mechanism of BRONJ still remains unclear. Further studies are necessary to evaluate the detailed mechanism of BRONJ development.

4.2 Denosumab

Denosumab is an OC inhibitor that plays a role in the supportive care of MM OBD. It is a monoclonal antibody, given subcutaneously, that inhibits OC activity through targeting RANKL. Denosumab is approved for increasing bone density in patients with osteoporosis and for preventing SREs in patients with metastatic bone disease [100]. It has been recently reported that denosumab causes osteosclerosis [101], and hypercalcemia has been observed following discontinuation of denosumab [102] in children. In MM, although a favorable trend was observed, denosumab was

equivalent to ZA in delaying time to first on-study SRE [103]. Denosumab is not currently FDA approved for use in patients with MM; a larger, ongoing phase III study (ClinicalTrials.gov identifier: NCT01345019) is comparing it with ZA in this disease setting.

4.3 OPG Agonists

OPG is a decoy receptor for RANKL, and it blocks OC differentiation and activation. In MM patients, BM plasma levels of OPG is decreased compared with normal volunteers and patients with MGUS [35]. Importantly, low levels of OPG in serum correlate with advanced OBD in MM [55]. Treatment with OPG or OPG-like molecules prevented both bone destruction and MM growth in vivo [36, 56]. A Phase I study of a recombinant OPG construct (AMGN-0007) was conducted in MM patients with OBD, and decreased NTX/creatinine levels was observed [104].

4.4 CCR1 Inhibitors

The CCL3/CCR1 pathway stimulates OC differentiation, MM cell survival and migration, and inhibits OB differentiation suggesting that CCL3/CCR1 is a relevant target in MM OBD. Both antisense sequence and neutralizing antibody against CCL3 effectively inhibited tumor growth and restored bone remodeling in a mouse model of MM OBD [15, 105]. Similar results have been shown with a clinical grade small molecule CCR1 antagonist, MLN3897 (Millennium Pharmaceuticals) [51]. In addition to these molecules, several CCR1 antagonists were evaluated for MM OBD [106, 107]. Future clinical trials using CCR1 inhibition strategies in patients with MM OBD will help to confirm these promising preclinical results.

4.5 Anti-BAFF—Neutralizing Antibody

In MM, BAFF is expressed by monocytes, macrophages, dendritic cells, T cells, neutrophils, MM cells, and OCs [65, 108–111]. BAFF is a MM cell survival factor and rescues MM cells from apoptosis induced by IL-6 deprivation and dexamethasone via activation of NF-kB, PI-3 kinase/AKT, and MAPK kinase pathways and induction of a strong upregulation of the Mcl-1 and Bcl-2 antiapoptotic proteins [65]. In vivo—neutralizing antibodies against BAFF (LY2127399, Eli Lilly) significantly inhibit tumor burden and, importantly, reduce OBD and OC differentiation in preclinical setting [66]. On the basis of these results, a clinical trial combining BAFF-neutralizing antibody with proteasome inhibitor, bortezomib is currently ongoing, preliminary results from Raje et al. reported the treatment was well tolerated and 22 of the 48 patients enrolled achieved a partial remission or better (https://ash.confex.com/ash/2012/webprogram/Paper52052.html).

4.6 Activin A Antagonists

Activin A is secreted by BMSCs and OCs in MM OBD. Activin A stimulates OC differentiation and inhibits OB formation in MM OBD. Activin A can be targeted by a chimeric antibody RAP-011 (Acceleron Pharma), derived from the fusion of the extracellular domain of type II activin receptor (ActRIIA) and the constant domain of the murine IgG2a [112]. RAP-011 enhances OB mineralization and increases bone density in an osteoporotic mouse model. In MM, RAP-011 reversed OB inhibition, improved MM bone disease, and inhibited tumor growth in an in vivo humanized MM model [9]. In human, ACE-011 which is the humanized counterpart of RAP-011 effectively decreased bone resorption markers, C-terminal type 1 collagen telopeptide (CTX) and TRACP-5b and increased bone formation marker, serum levels of bone-specific alkaline phosphatase (BSALP) in post-menopausal women [113]. It has been shown in vitro that lenalidomide, a well known and approved treatment strategy for relapsed MM, stimulates activin A secretion on BMSCs via an Akt-mediated increase in JNK signaling [14]. Clinical trials for ACE-011 with Lenalidomide + Dexamethasone are ongoing and evaluating its role in MM (ClinicalTrials.gov identifier: NCT01562405).

4.7 Dkk-1 Antagonists

Dkk-1 plays one of the key roles in mediating OB inhibition in MM [71]. Therefore, treatment strategies to block Dkk-1 activity have been developed. In vitro assays show that inhibition of Dkk-1 via a specific neutralizing antibody promotes OB differentiation and function and reverses the negative effect of MM cells on OB differentiation [114, 115]. Moreover, in vivo studies using both murine and humanized murine models of MM-induced bone disease showed increased bone formation, OB numbers, and improvement of osteolytic lesions by Dkk-1 inhibition [115–117]. Importantly, blocking Dkk-1 also resulted in reduction of tumor growth, mainly as an indirect effect via modification of the tumor microenvironment [115]. Therefore, Dkk-1 inhibition via a neutralizing antibody restores bone homeostasis and may have an inhibitory effect on tumor growth. Currently, ongoing clinical trials combining Dkk-1 neutralizing antibody and bisphosphonates will test these promising preclinical results. In particular, ZA in combination with the proanabolic agent BHQ880, a fully human anti-Dkk-1 monoclonal antibody, has being studied in a phase I clinical trial (ClinicalTrials.gov identifier: NCT00741377). BHQ880 was also tested in a phase II clinical trial in smoldering MM (ClinicalTrials.gov identifier: NCT01302886) and preliminary results showed that BHQ880 significantly stimulated the vertebral strength by qCT from a baseline of 3 % ($P = 0.002$) (https://ash.confex.com/ash/2012/webprogram/Paper48568.html).

4.8 Sclerostin Neutralizing Antibody

Several studies have already demonstrated the importance of sclerostin in osteoporosis [118, 119], and inhibition of sclerostin represents an important strategy in the treatment of bone conditions with high catabolism. In fact, clinical trials with sclerostin neutralizing antibodies, romosozumab and blosozumab for the treatment of postmenopausal osteoporosis are ongoing and preliminary results have shown increase of bone mineral density [120–122]. In MM, higher circulating levels of sclerostin have been found in newly diagnosed MM patients, and it correlated with MM disease stage and fractures [74]. These data underscore the importance of targeting sclerostin for treatment of MM OBD. However, the source and role of sclerostin in MM OBD still remains unclear. Further studies about sclerostin's role in MM and application of sclerostin neutralizing antibody to MM OBD are expected.

4.9 Bortezomib

Bortezomib is a proteasome and NF-kB signaling pathway inhibitor with potent anti-MM activity. Bortezomib also inhibits MM-BMSC interactions, impairs osteoclastogenesis, and stimulates mesenchymal stem cell differentiation to OB and, therefore, actively modulates bone remodeling in MM [123–125]. The anabolic effects of bortezomib are associated with Runx2 upregulation via inhibition of proteasomal degradation. Runx2 is a critical transcription factor in early OB differentiation and modulates the expression of the OB-specific transcription factor osterix [125, 126]. The anti-OC effects of bortezomib are mediated by p38 inhibition at early time points and, at later time points, by impairment of NF-kB signaling and AP1 inhibition [123]. These effects have been confirmed in the clinical setting by upregulation of OB activation markers (BSALP and osteocalcin) and downregulation of bone resorption markers (CTX and TRACP-5b) as well as decrease of Dkk-1 and sRANKL in patients treated with bortezomib [127].

4.10 Carfilzomib

In contrast to bortezomib, carfilzomib is a new proteasome inhibitor that is associated with a very low incidence of peripheral neuropathy. Carfilzomib is a structural analog of the microbial natural product epoxomicin that selectively inhibits the chymotrypsin-like activity of both the constitutive proteasome and the immunoproteasome [128]. It was recently approved in July 2012 for patients with MM experiencing disease progression after prior therapy with bortezomib and an immunomodulatory drug. Carfilzomib strongly stimulates OB calcification and inhibits OC differentiation in addition to the antitumor effect [129–131]. Moreover, we showed carfilzomib reversed OB inhibition, improved MM bone disease, and inhibited tumor growth in an in vivo disseminated MM model [131]. Interestingly,

we could not see upregulation of OB differentiation marker in OBs in the presence of higher concentration of carfilzomib although the concentration of carfilzomib strongly stimulates OB calcification. Further studies are necessary to evaluate the detailed mechanism of carfilzomib effect on OBs.

4.11 Bruton's Tyrosine Kinase Inhibitors

Bruton's tyrosine kinase (Btk) belongs to the Tec family of tyrosine kinases. The activation of Btk regulates B-cell development and antibodies production. Thus, Btk pathway is a potential therapeutic target in a variety of B-cell malignancies, including Waldenström's macroglobulinemia, diffuse large B-cell lymphoma, follicular lymphoma, mantle cell lymphoma and chronic lymphocytic leukemia [132]. In MM, we showed that Btk inhibitor, CC-292 strongly inhibits OC activity and improves MM OBD [131]. It decreased only INA-6 MM cell line viability in higher concentration, however, had negligible direct in vitro effects on other MM cells viability or in animal models. On the other hand, the other Btk inhibitors, PCI-32765 (ibrutinib) and LFM-A13 have shown to display some antitumor effect in MM xenograft mouse model when INA-6 MM cells were used [133, 134]. More investigations are needed to reveal the role of Btk inhibitors in the MM OBD.

4.12 Pim Inhibitor

MM cells upregulate Pim-2 expression in BMSCs/OBs and inhibit OB differentiation [69]. Meanwhile, IL-6, produced by BMSCs, BAFF, and APRIL, produced by OCs, stimulate Pim-2 expression in MM cells via activation of NF-κB and JAK2/STAT3 pathway, resulting in MM cell survival [67]. Importantly, Pim inhibitor prevents bone destruction while suppressing MM tumor burden in MM model mouse [69]. Pim-2 may become a new target for not only MM OBD but also MM treatment.

5 Conclusion

Our understanding of the biology of MM OBD was remarkably advanced in these decades. Although OCs are a critical player in the pathogenesis of bone disease, other BM microenvironmental cells such as osteocytes, OBs, and BMSCs are affected in MM and contribute to the development of MM OBD. Many novel targets for MM OBD have been discovered following these insights. Effective therapeutic strategies to overcome MM-induced OBD should target the osteocyte-OB-OC axis, combining bone-anabolic with anticatabolic agents. Such novel agents for MM OBD restoring bone balance in MM represent a novel strategy to overcome osteolytic disease and, more provocatively, to create a hostile niche for

MM tumor growth. Although there are still many unknown parts in MM OBD, further investigations will reveal these and a wide range of targeted therapies may become available to treat MM OBD more effectively in the near future.

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