Published in final edited form as:

Bone. 2016 May ; 86: 131–138. doi:10.1016/j.bone.2016.02.019.

The effects of proteasome inhibitors on bone remodeling in multiple myeloma

Maurizio Zangari^{a,*} and Larry J. Suva^{b,1}

^aMyeloma Institute, University of Arkansas for Medical Sciences, Little Rock, AR, USA

^bDepartment of Orthopedic Surgery, Center for Orthopaedic Research, University of Arkansas for Medical Sciences, Little Rock, AR, USA

Abstract

Bone disease is a characteristic feature of multiple myeloma, a malignant plasma cell dyscrasia. In patients with multiple myeloma, the normal process of bone remodeling is dysregulated by aberrant bone marrow plasma cells, resulting in increased bone resorption, prevention of new bone formation, and consequent bone destruction. The ubiquitin–proteasome system, which is hyperactive in patients with multiple myeloma, controls the catabolism of several proteins that regulate bone remodeling. Clinical studies have reported that treatment with the first-in-class proteasome inhibitor bortezomib reduces bone resorption and increases bone formation and bone mineral density in patients with multiple myeloma. Since the introduction of bortezomib in 2003, several next-generation proteasome inhibitors have also been used clinically, including carfilzomib, oprozomib, ixazomib, and delanzomib. This review summarizes the available preclinical and clinical evidence regarding the effect of proteasome inhibitors on bone remodeling in multiple myeloma.

Keywords

Bone remodeling; Proteasome inhibitor; Multiple myeloma; Osteoblast; Osteoclast; PTH

1. Introduction

Bone disease, defined as the presence of at least 1 osteolytic bone lesion or diffuse osteoporosis with compression fractures, is a characteristic feature of multiple myeloma (MM) [1]. Bone disease occurs in MM because of dysregulated bone remodeling, a process in which MM cells interacting with the bone marrow microenvironment disrupt the normal balance between bone resorption and bone formation [2,3]. This disruption of bone homeostasis results in the prevention of new bone formation and leads to bone destruction [2,3].

*Corresponding author at: Myeloma Institute, University of Arkansas for Medical Sciences, 4301 W. Markham St #721-4, Little Rock, AR 72205, USA. MZangari@uams.edu (M. Zangari).

¹Current address, Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX, USA.

Conflict of interest MZ has received research funding from Onyx and Millenium. LS has no conflicts to disclose.

As a consequence of altered bone remodeling, up to 90% of patients with MM develop bone lesions that can, in turn, cause a sequelae of skeletal-related events (SREs) such as bone pain, pathologic fractures, spinal cord compression, and hypercalcemia [2,4]. The occurrence of SREs has been linked to inferior survival [5,6], reduced quality of life [7], and increased healthcare costs for patients with MM [8,9].

Treatment with bisphosphonates is the current standard of care for the management of myeloma-related bone disease and is recommended for all patients with MM receiving frontline therapy [9]. Bisphosphonates inhibit osteoclasts and thereby prevent bone resorption [10]. Although bisphosphonates have been shown to reduce the incidence and severity of SREs, improve quality of life, and prolong survival (in the case of zoledronic acid) compared with placebo [9], these agents do not restore bone formation [11]. In addition, bisphosphonates have been associated with renal toxicities and osteonecrosis of the jaw, which may limit their long-term use [2,10]. These drawbacks have spurred investigations into other agents that could simultaneously prevent bone resorption and promote bone formation, while also being safe and tolerable [10,11].

There is evidence that proteasome inhibition may be an effective strategy to improve bone remodeling in patients with MM. In contrast to bisphosphonate therapy, proteasome inhibition has been found to simultaneously inhibit bone resorption and promote bone formation [11–13]. The first-in-class proteasome inhibitor bortezomib and the next-generation proteasome inhibitor carfilzomib are established anti-MM agents, having been already approved for use in treating patients with MM. Preclinical and clinical data demonstrate that bortezomib has significant beneficial effects on bone metabolism [2,11,14–17]. Agents such as proteasome inhibitors, which combine anti-MM activity with improved bone remodeling, may be a particularly attractive treatment option for patients with myeloma-related bone disease [14]. Herein, we review data from preclinical and clinical studies that have examined the effects of bortezomib and next-generation proteasome inhibitors on bone remodeling in patients with MM.

2. Overview of abnormal bone remodeling in MM

The structure and integrity of the skeleton are maintained by a tightly coordinated process of bone remodeling [18,19]. In this process, osteoclasts continually resorb damaged bone, which is replaced by new bone synthesized by osteoblasts [18–20]. The tight control of bone resorption, once thought to be driven by osteoblasts, has now been shown to be the domain of osteocytes, the most abundant cells in bone that are encased in the mineralized matrix [21,22]. The process of bone remodeling and bone homeostasis is disrupted in patients with MM. Either directly or through complex interactions with the bone marrow microenvironment, MM cells stimulate the bone-resorptive activity of osteoclasts and suppress the bone-forming activity of osteoblasts, ultimately causing significant bone destruction [2,3,23]. The control of both resorption and formation is the realm of the osteocyte and it seems likely that these cells are also dysregulated in myeloma [24].

In recent years, key signaling pathways and cytokines that regulate osteoclast and osteoblast activity, both in normal and abnormal bone remodeling, have been identified and elucidated,

as reviewed by Zangari et al., Silbermann and Roodman, Raje and Roodman, and Terpos et al. [2,3,11,23]; importantly, MM cells have been found to dysregulate many of these pathways. Under normal conditions, the coordinated and balanced signaling between the receptor activator of NF- κ B (RANK), its ligand RANKL, and the decoy receptor of RANKL, osteoprotegerin (OPG), helps to maintain healthy levels of osteoclast activity and bone resorption [3,23,24]. The binding of RANKL to RANK promotes the formation, activation, and survival of osteoclasts; in contrast, the blockade of RANKL–RANK binding by OPG inhibits the activity of osteoclasts [3,23,25]. MM cells disrupt the RANK/RANKL/OPG axis by enhancing RANKL expression, promoting the binding of RANKL with RANK, and by decreasing the expression of OPG [3,23,25]. Patients with MM have an increased ratio of RANKL to OPG [26,27], the magnitude of which has been found to correlate with markers of bone resorption and the number of osteolytic bone lesions [27].

In addition to RANK, the expression and/or activity of many other osteoclast-activating factors are altered by MM cells including parathyroid hormone-related protein (PTHrP) [28], activin [29], macrophage inflammatory protein-1 α [30], macrophage colony-stimulating factor [31], monocyte chemoattractant protein 1 [32], stromal derived factor-1 α [33], tumor necrosis factor- α [34,35], and interleukin-3 [36]. MM cells may also affect the activity of osteoclasts through their deregulation of the Notch signaling pathway, as pharmacologic inhibition of the Notch pathway has been found to result in the abrogation of RANKL-induced osteoclast differentiation in vitro and a reduction in the number of osteolytic lesions in a murine model of MM [37].

The differentiation and activity of bone-forming osteoblasts are primarily mediated by the transcription factor runt-related transcription factor 2 (Runx2)/core-binding factor subunit alpha-1 (Cbfa1) and the wingless-type (Wnt) signaling pathway [2,23]. MM cells have been shown to down-regulate Runx2/Cbfa1 activity in osteoblast progenitor cells, which has been shown to suppress the formation and differentiation of osteoblasts [38]. Interestingly, MM cells themselves have been demonstrated to have a higher level of Runx2 expression than normal plasma cells; this overexpression of Runx2 in MM cells has been associated with tumor growth and bone metastasis [39]. In addition, it has been demonstrated that MM cells inhibit the Wnt pathway by overexpressing or producing Wnt inhibitors such as Dickkopf-1 (DKK-1) [40], secreted frizzled related proteins [41], and sclerostin [42]. The serum concentrations of DKK-1 and sclerostin have been correlated with the level of abnormal bone modeling in patients with MM [42,43].

An additional bone cell shown to play a key role in abnormal bone remodeling is the osteocyte. Osteocytes are osteoblast-derived cells that have become embedded within mineralized bone matrix [44]. In one study, patients with MM who had bone lesions were found to have significantly fewer viable osteocytes compared with healthy controls and patients with MM who did not have bone lesions [45]. This study demonstrated that MM cells induced apoptosis in osteocytes and altered their transcriptional profiles, resulting in the up-regulation of osteoclastogenic cytokines [45]. In patients with MM, apoptotic osteocytes may also reduce bone formation through their secretion of sclerostin, an inhibitor of Wnt-dependent osteoblast differentiation and survival [46].

3. Proteasome inhibitors and bone remodeling in MM

Proteasome inhibitors are hypothesized to normalize bone remodeling in patients with MM based on research demonstrating that the ubiquitin-proteasome system controls the catabolism of several proteins (e.g. I κ B [13], bone morphogenetic proteins [47], Jak1 [48], zinc-finger transcription factor Gli2 [49], β -catenin [50], and DKK-1 [51]) that regulate bone remodeling (Fig. 1) [2,25]. Supporting this hypothesis, early preclinical studies demonstrated that proteasome inhibition reduced bone resorption and increased bone formation [12,13,52].

3.1. Bortezomib

The results described earlier provided the impetus to investigate the effect of bortezomib, a boronate-based proteasome inhibitor, on bone remodeling in preclinical models of myeloma-related bone disease and in patients with MM. To date, the effect of bortezomib on bone remodeling in MM has been extensively investigated in the preclinical and clinical settings (Tables 1 and 2). The results from these investigations have recently been reviewed by Qiang et al., Zangari et al., Terpos et al., and Mohty et al., [2,14,15,25] and are summarized below.

In preclinical models, bortezomib has been shown to have varied and substantial effects on the activities of osteoclasts, osteoblasts, and osteocytes. Studies have found that bortezomib inhibits the differentiation of osteoclasts [54,55], decreases the resorption capacity of osteoclasts [54], reduces the total number of functional osteoclasts [56], and prevents the death of osteocytes [57]. These effects were mediated by inhibition of p38 mitogen-activated protein kinase pathways, down-regulation of tumor necrosis factor 6, and blockade of p38, AP-1, NF- κ B activation [54,55], and modulation of autophagy [57].

Preclinically, bortezomib induces osteoblast differentiation by up-regulation of Runx2/Cbfa1 activity in human osteoblast progenitors and osteoblasts without affecting nuclear and cytoplasmic active β -catenin levels. Bortezomib also increases osterix, vitamin D receptor signaling, and Gli-3 [58–61] and by inhibition of DKK-1 expression [50,51] and fibroblast growth factor 2-induced reduction of transcriptional coactivator with PDZ-binding motif levels in osteoblast-like cells [62]. Bortezomib treatment also enhances the osteogenic differentiation capacity of mesenchymal stromal cells, which have the ability to differentiate into osteoblasts and possibly regulate E3 ubiquitin ligase Smurf1 (Smad ubiquitin regulatory factor 1) degradation [60,63–64].

Based on the molecular mechanisms of abnormal bone remodeling in MM, the effects of bortezomib on osteoclasts, osteoblasts, and osteocytes would be expected to improve bone formation, and this is indeed what has been observed in animal models of MM. Bone mineral density, trabecular number, and trabecular bone volume have all been reported to be increased with bortezomib treatment in murine models of MM when compared with treatment without bortezomib [56,65]. In addition, a significant decrease in the number of osteolytic lesions has been observed with bortezomib treatment in murine models of MM [56].

The preclinical results summarized earlier provided the foundation for clinical studies evaluating the effect of bortezomib on bone remodeling in patients with MM and bone disease. Clinical evidence for the beneficial effects of bortezomib (used as a single agent or in combination therapy) on bone remodeling has emerged from the analyses of bio-markers of bone metabolism and from direct measurements of bone characteristics performed in patients with newly diagnosed or relapsed/refractory MM.

As reviewed by Zangari et al., the majority of clinical studies have demonstrated that treatment with bortezomib is associated with an increase in the levels of biomarkers associated with bone formation and a reduction in the levels of biomarkers associated with bone resorption [2]. For example, bortezomib-based treatment has been reported to be associated with increased levels of serum alkaline phosphatase (ALP), bone ALP, and serum parathyroid hormone (PTH; markers of bone formation) [66–69], as well as reduced levels of DKK-1 (a marker of bone resorption) [66,70,71]. A number of studies have indicated that changes in some of these biomarkers are present in patients who do and do not respond to therapy with bortezomib. In one study, the levels of bone ALP and osteocalcin were found to be significantly increased in both responders and nonresponders to bortezomib-based therapy, as compared with those in patients who did not receive a bortezomib-based regimen [71]. The drug specific bone effect was also suggested by the observation that serum alkaline phosphatase was not changed in dexamethasone responding patients during the Apex Trial [69].

In contrast, other studies have demonstrated an association between the response to bortezomib and changes in the level of certain bone biomarkers. For example, a rapid increase in serum concentrations of PTH was observed in patients who achieved a partial response or better after treatment with bortezomib, but not in patients who achieved less than a partial response [68]. This observation suggested that the anti-MM activity of bortezomib may depend on the presence of PTH and/or its receptors. Supporting this hypothesis, the anti-MM activity of bortezomib was shown to be mediated by PTHR1; concomitant treatment with a PTHR1 antagonist was found to abrogate the antitumor effect of bortezomib in a mouse model of MM [72]. Preliminary evidence also indicates that a higher expression level of PTHR1 or PTHR2 in bone marrow is associated with longer overall survival in patients with newly diagnosed MM who were enrolled in Total Therapy 3 (where bortezomib is part of the treatment regimen) [73,74]. The underlying mechanisms driving the rapid increments in serum PTH levels are unknown. Interestingly, we have observed stable serum calcium levels despite the elevated serum PTH serum [Zangari unpublished data]. Unfortunately, widespread clinical use of PTH in myeloma patients is unacceptable given the black box labeling preventing PTH use in patients with cancer. In addition, based on the increased bone mass observed after treatment with modest anti-resorptive agents (such as cathepsin k inhibitors), it is now well-accepted that even the modest resorption inhibition can result in increased bone mass that is disproportionate to that of resorption inhibition alone. Indeed, even anabolic agents such as PTH have been shown to elicit compartment-specific differences in anabolism [75–76]. Taken together, these data suggest that bortezomib inhibits bone resorption and promotes bone formation through a complex interplay of direct effects on bone remodeling and indirect effects associated with its anti-MM activity.

Supporting this biomarker evidence, imaging studies and analyses of clinical bone disease events have demonstrated that bortezomib has beneficial effects on bone health in patients with MM. Relative to baseline levels, increased bone mineral density, bone matrix deposition, bone volume, and trabecular thickness in patients with MM have been associated with bortezomib treatment [14,68,77,78]. Although the number of patients is limited, it is important to note that evidence of bone healing (determined from radiographic imaging) has been reported in 50% (13/26) to 55% (6/11) of patients with MM who were treated with bortezomib [66,79]. In the randomized phase 3 VISTA trial that evaluated bortezomib plus melphalan and prednisone (VMP) versus melphalan plus prednisone (MP) in patients with newly diagnosed MM, rates of bisphosphonate use (73% vs 82%), progression of worsening bone disease (3% vs 11%), and the need for radiation therapy (3% vs 8%) were lower with VMP compared with MP [66]. Notably, a recent study that evaluated bortezomib plus thalidomide and dexamethasone (VTD) as consolidation therapy in patients with newly diagnosed MM demonstrated that VTD consolidation was associated with RANKL/OPG ratio normalization achieving a very low incidence of SREs (2%) which was obtained despite the absence of concomitant bisphosphonate therapy [80]. Further studies are needed to evaluate whether treatment with bortezomib obviates the need for bisphosphonate therapy in patients with newly diagnosed MM.

The promising results obtained with bortezomib for the treatment of myeloma-related bone disease have spurred investigations into the effects of second-generation proteasome inhibitors on bone remodeling in MM (Table 3). In the following sections, we review the preclinical and clinical data that have emerged, suggesting that these newer proteasome inhibitors have bone remodeling effects similar to those of bortezomib.

3.2. Carfilzomib and oprozomib

Carfilzomib and oprozomib are 2 epoxyketone-based proteasome inhibitors found to affect bone remodeling. Carfilzomib is a proteasome inhibitor approved for use as a single agent for the treatment of patients with relapsed and refractory MM. Oprozomib is an orally bioavailable proteasome inhibitor similar to, but distinct from carfilzomib. In preclinical studies, carfilzomib and oprozomib were shown to have antiresorptive and bone-anabolic activity similar to that of bortezomib [81]. They strongly inhibit the differentiation and formation of osteoclasts; in one study, osteoclastogenesis was reduced by 50% with these agents [81]. In addition, carfilzomib and oprozomib inhibit the osteoclastic bone resorption via the disruption of RANKL-induced NF- κ B activation and suppress osteoclast stimulation by decreasing osteoblast expression of RANK [81].

In terms of bone-anabolic activity, carfilzomib and oprozomib promote the differentiation of osteoblasts from mesenchymal stem cells (similar to bortezomib) [63], and increase matrix mineralization and calcium deposition in vitro [81]. Carfilzomib induces osteoblast differentiation via Wnt-dependent activation of the β -catenin/T-cell factor pathway [82] and stimulates osteogenesis via inhibition of the notch1 receptor (part of the Notch signaling pathway) [83]. In murine models of MM, treatment with carfilzomib or oprozomib was associated with a reduction of tumor-induced bone loss [81]. Notably, it was observed that carfilzomib was consistently more effective than bortezomib in enhancing osteoblast activity

in vitro and in vivo, suggesting that carfilzomib may have a more potent effect on bone formation compared to that of bortezomib [81]. The mechanisms underlying this observation are currently unknown and warrant further study.

As with bortezomib, PTHR1 has been shown to mediate the anti-MM effect of carfilzomib treatment in a mouse model of MM [72]. Preclinically, carfilzomib has been shown to block PTH-induced osteoclastogenesis via inhibition of the proteasomal degradation of HDAC4 in osteoblasts and disruption of RANKL-induced NF- κ B activation in osteoclasts [84]. Given these findings, the relationship between the PTH/PTHr1 axis and the bone formation properties of carfilzomib also merits further study in patients with MM.

Preclinical studies also suggest that carfilzomib in combination with other novel agents, such as a Bruton's tyrosine kinase inhibitor, may possess synergistic activity against bone disease. In a murine model of MM, treatment with the combination of carfilzomib and CC-292, a novel Bruton's tyrosine kinase inhibitor, increased trabecular bone thickness and bone volume to a greater degree than either agent alone [85]. To date, there have been no prospective clinical studies evaluating the effects of carfilzomib or oprozomib on bone remodeling. However, in a retrospective analysis of 2 phase 2 studies of single-agent carfilzomib in relapsed and/or refractory MM (PX-171-003 and PX-171-004), an early elevation in the levels of ALP was found to be associated with subsequent response [86].

3.3. Ixazomib

Ixazomib (MLN9708) is a next-generation oral, boronate-based proteasome inhibitor with demonstrated anti-MM activity. There has been one preclinical study investigating the effect of this agent on myeloma-related bone disease [87]. In this study, ixazomib was found to inhibit in vitro osteoclastogenesis and resorption [87]. Akin to the effects of other proteasome inhibitors, the effects of ixazomib on osteoclasts were partially mediated by inhibition of RANKL-induced NF- κ B signaling [87].

Ixazomib was also shown to stimulate osteogenic differentiation of mesenchymal cells in vitro and promote osteoblast function and matrix mineralization [87]. Similar to the mechanisms observed for carfilzomib, the effects of ixazomib on osteoblasts were linked with enhanced β -catenin/T-cell factor signaling [87]. In a murine model of MM, ixazomib treatment was associated with increased trabecular bone volume and connectivity as well as reduced trabecular separation compared with control [87]. Ixazomib appears to be at least as effective as bortezomib in the prevention of myeloma-related bone loss [87].

3.4. Delanzomib

Delanzomib is a novel orally active, boronate-based proteasome inhibitor that targets the chymotrypsin-like activity of the proteasome. Data regarding the bone remodeling properties of delanzomib are limited, with one study having investigated the effect of this agent on osteoclasts [88]; delanzomib to be a potent suppressor of RANKL-mediated osteoclastogenesis in vitro [88]. Notably, delanzomib was shown to inhibit osteoclast formation to a greater degree than bortezomib ($p < 0.003$) [87]. As noted previously with carfilzomib, the mechanisms underlying the more potent effects of next-generation proteasome inhibitors such as delanzomib on bone remodeling merit additional study.

4. Conclusions and future directions

Taken together, the findings reviewed herein suggest that proteasome inhibitors as a class substantially influence bone remodeling in MM. Bortezomib, along with next-generation proteasome inhibitors, modify the bone microenvironment from a catabolic state to an anabolic one [81]. Importantly, there is preliminary evidence that next-generation proteasome inhibitors may have even more potent effects on bone remodeling compared with those associated with the use of bortezomib.

Since the evidence suggests that proteasome inhibitors have substantial and beneficial effects on bone remodeling, targeting proteasome inhibitors to bone may be an advantageous therapeutic strategy [89]. The combination of proteasome inhibitors with immunomodulators (such as structural and functional analogues of thalidomide) has a robust rationale that has been shown prospectively to reduce high dose chemotherapy-associated bone resorption resulting in a low incidence of skeletal related events [89]. In addition, the conjugation of proteasome inhibitors to bisphosphonates and loading proteasome inhibitors into bone-specific nanoparticles are recently proposed methods to achieve bone-targeted delivery of proteasome inhibitors [90,91]. These methods are under active preclinical development and, if successful, may enable an even more effective treatment of myeloma-related bone disease.

Data from prospective, randomized studies will help to further clarify the role of proteasome inhibitors in bone remodeling. In particular, analyses of markers of bone remodeling from the randomized phase 3 FOCUS trial comparing single-agent carfilzomib with low-dose corticosteroids and optional cyclophosphamide in patients with relapsed and refractory MM will provide additional information regarding the clinical effectiveness of carfilzomib in improving bone formation and mitigating bone disease. We eagerly await the results of these and other ongoing investigations, since such treatment regimens would have profound consequences for the treatment of myeloma bone disease and for the quality of life of these patients.

Acknowledgments

Medical writing and editorial assistance were provided by BlueMomentum, an Ashfield Company, part of UDG Healthcare plc, and funded by Onyx Pharmaceuticals, Inc., an Amgen subsidiary.

Abbreviations

ALP	alkaline phosphatase
Cbfa1	core-binding factor subunit alpha-1
DKK	dickkopf
MM	multiple myeloma
MP	melphalan with prednisone
OPG	osteoprotegerin

PTH	parathyroid hormone
PTHrP	parathyroid hormone-related protein
PTHr1	parathyroid hormone 1 receptor
RANK	receptor activator of nuclear factor- κ B
RANKL	receptor activator of nuclear factor- κ B ligand
Runx2	runt-related transcription factor 2
SRE	skeletal-related event
Wnt	wingless-type
VMP	bortezomib with melphalan and prednisone
VTD	bortezomib with thalidomide and dexamethasone

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Up or down-regulated in MM
Increased/stimulated or decreased/inhibited by proteasome inhibition

Fig. 1.

Signaling between bone cells and potential mechanisms of action of proteasome inhibitors for myeloma-related bone disease. BMP = bone morphogenetic protein, BMPR = bone morphogenetic protein receptor, DKK-1 = Dickkopf-1, FZD = frizzled, LRP5/6 = lipoprotein receptor-related protein, MM = multiple myeloma, NFATc1 = nuclear factor of activated T-cells, cytoplasmic 1, OPG = osteoprotegerin, PKA = protein kinase A, PTHr1 = parathyroid hormone 1 receptor, RANK = receptor activator of nuclear factor- κ B, RANKL = RANK ligand, ROR2 = receptor tyrosine kinase-like orphan receptor 2, WNT = wingless type. Adapted with permission from Macmillan Publishers Ltd.: Nature Medicine [53], copyright 2013.

Table 1

Impact of bortezomib on bone remodeling: evidence from select preclinical studies.

Reference	Samples	Effect of bortezomib on bone remodeling
Hongming and Jian [54]	PBMCs from patients with MM	• Reduced osteoclast differentiation and resorption capacity
von Metzler et al. [55]	Primary human osteoclasts from CD14+ precursors	• Reduced osteoclast differentiation and resorption capacity
Deleu et al. [56]	5T2MM murine model of MM	<ul style="list-style-type: none"> • Reduced number of osteoclasts • Increased number of osteoblasts • Reduced number of osteolytic bone lesions • Increased trabecular number and trabecular bone volume
Toscani et al. [57]	Murine osteocytic cell line MLO-Y4; human pre-osteocytic one HOB-01	• Increased viability of osteocytes
Giuliani et al. [59]	Human osteoblast progenitors and osteoblasts	• Increased osteoblast differentiation in human mesenchymal stem cells
Qiang et al. [50]	Osteoprogenitor cells and primary mesenchymal stem cells	• Induction of osteoblast differentiation
Sanvoranart et al. [63]	Human mesenchymal stem cells from bone marrow and postnatal sources	• Enhancement of osteogenic differentiation of mesenchymal stem cells
Pennisi et al. [65]	SCID-rab mice engrafted with MM cells from human patients	<ul style="list-style-type: none"> • Increased bone mineral density • Increased number of osteoblasts • Reduction in the number of osteoclasts

MM = multiple myeloma, PBMC = peripheral blood mononuclear cell, SCID = severe combined immunodeficiency disease.

Table 2

Impact of bortezomib on bone remodeling: evidence from select clinical studies in MM.

Reference	Treatment	Effect of bortezomib-based treatment on bone remodeling
Zangari et al. [69]	Bortezomib ± dexamethasone	• Increased levels of ALP, bALP, and PTH in patients who responded to bortezomib
Zangari et al. [68]	Bortezomib	• Increased levels of ALP, PTH, and osteocalcin in patients who responded to bortezomib
Heider et al. [71]	Bortezomib ± dexamethasone	• Increased levels of bALP and osteocalcin in patients who did and did not respond to a bortezomib-based regimen
T'Seyen et al. [79]	Bortezomib-based regimens	• Evidence of bone healing in 13/26 (50%) of patients who were treated with bortezomib
Delforge et al. [66]	VMP vs MP	<ul style="list-style-type: none"> • Median maximum ALP increase was significantly higher with VMP vs MP overall (49.7% vs 30.3%, $p = 0.029$) • Greater maximum ALP increase was significantly associated with achievement of a CR • Fewer bone disease events and improvements in bone remodeling with VMP vs MP
Terpos et al. [80]	VTD	• Low incidence of SREs (2%)

ALP = alkaline phosphatase, bALP = bone alkaline phosphatase, CR = complete response, MM = multiple myeloma; MP = melphalan with prednisone, PTH = parathyroid hormone, SRE = skeletal-related event, VMP = bortezomib with melphalan and prednisone, VTD = bortezomib with thalidomide and dexamethasone.

Table 3

Effect of second-generation proteasome inhibitors on bone remodeling: evidence from preclinical studies.

Reference	Treatment	Samples	Effect of treatment on bone remodeling
Hurchla et al.[81]	Carfilzomib or oprozomib	<ul style="list-style-type: none"> • Osteoclasts and mesenchymal stem cells of human and murine origin • Murine model of disseminated MM 	<ul style="list-style-type: none"> • Inhibited osteoclast formation and bone resorption in vitro • Enhanced osteogenic differentiation and matrix mineralization in vitro • Increased trabecular bone volume, decreased bone resorption, enhanced bone formation, and prevented bone loss in vivo
Hu et al. [82]	Carfilzomib	• Osteoprogenitor cells and primary mesenchymal stem cells from patients with MM	• Promoted mesenchymal stem cell differentiation into osteoblasts via Wnt-independent activation of the β -catenin/TCF pathway
Li et al. [83]	Carfilzomib	• Mesenchymal stem cells	• Stimulated osteogenesis via inhibition of notch1
Yang et al. [84]	Carfilzomib	• Cell cultures	• Suppressed PTH-induced osteoclastogenesis via the blockade of proteasomal degradation of HDAC4 in osteoblasts and the inhibition RANKL-mediated I κ B degradation in osteoclasts
Eda et al. [85]	Carfilzomib with CC-292	<ul style="list-style-type: none"> • Osteoclasts from patients with MM • Mouse xenograft model of MM 	<ul style="list-style-type: none"> • Combination treatment inhibited osteoclast differentiation in vitro more than either agent alone • Combination treatment increased bone volume in vivo more than either agent alone
Garcia-Gomez et al. [87]	Ixazomib	<ul style="list-style-type: none"> • Osteoclasts and osteoclast precursors from patients with MM • Osteoprogenitors derived from bone marrow mesenchymal stem cells • Mouse model of bone marrow-disseminated MM 	<ul style="list-style-type: none"> • Inhibition of osteoclastogenesis and osteoclast resorption in vitro • Promotion of osteoblastogenesis and osteoblast activity in vitro • Augmented trabecular bone volume and connectivity in vivo
Piva et al. [88]	Delanzomib	• PBMCs from normal donors	• Suppression of osteoclastogenesis

HDAC = histone deacetylase, MM = multiple myeloma, PBMC = peripheral blood mononuclear cell, PTH = parathyroid hormone, RANK = receptor activator of nuclear factor kappa-B ligand, TCF = T-cell factor, Wnt = wingless-type.