Chapter 9
Osteoclasts: Potential Target for Blocking Microenvironmental Support of Myeloma

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Abstract Multiple myeloma (MM) bone disease is a major contributor to the morbidity and mortality of MM patients due to pathological fractures. The MM cells interact with the cells of the bone microenvironment to both generate bone lesions as a result of enhanced induction of osteoclastogenesis and prevent reactive new bone formation to heal the lesions by repressing osteoblast activity. The MM stimulated osteoclasts (OCLs) not only generate bone lesions, but also interact with the myeloma cells to promote the proliferation and survival of the MM cells through the generation of interleukin-6 (IL-6), osteopontin, fibroblast activation protein, BAFF, APRIL, and annexin II. These MM-supportive OCL products present therapeutic opportunities. Further, the enhanced bone resorption by OCLs releases immobilized growth factors from the bone matrix that both support the MM cells and further stimulate OCL differentiation in a vicious cycle. Hence, targeting osteoclast activity may inhibit myeloma growth. Therefore, bisphosphonates have been investigated for their anti-tumor affects. The MM cells increase osteoclast activity both directly and by stimulation of microenvironmental production of RANKL, MIP-1α, TNF-α and interleukins IL-1β, IL-3 and IL-6. These are therefore also possible therapeutic targets to inhibit myeloma bone disease.

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9.1 Introduction

Multiple myeloma (MM) is the most common cancer to involve bone with more than 80% of patients developing bone lesions [1]. The bone lesions are purely osteolytic in nature and do not heal in the vast majority of patients, even when they are in long-term complete remission. Up to 20% of patients will present with a fracture at diagnosis, 40% will sustain a pathologic fracture within the first year of diagnosis, and 60% of patients will develop pathologic fractures over the course of their disease [2]. MM bone disease is so severe because MM, like other osteolytic metastases, has increased osteoclastic bone destruction, but in contrast to other tumors, once MM tumor burden exceeds 50% in a local area, osteoblast activity is either severely depressed or absent [3].

Bone destruction in MM can involve any bone and is responsible for some of the most devastating aspects of the disease. The most common radiographic findings of bone involvement in MM are "punched-out" lytic lesions without reactive new bone formation and also include osteopenia, pathologic fractures, or a combination of these conditions. These findings demonstrate that enhanced osteoclast (OCL) activity is a major contributor to MM bone disease, which is further exacerbated by the suppressed osteoblast activity. This paradigm makes the OCL an attractive target for treating MM bone disease.

9.2 Role of the Osteoclast in Myeloma

The bone marrow microenvironment plays a pivotal role in the development of MM bone disease. Multiple factors are produced by both the MM cells and neighboring bone marrow stromal cells (BMSC) within the microenvironment, which interact to shift the normal delicate balance of bone destruction and new bone formation toward increased bone destruction with absent new bone formation. In addition, the OCL themselves play an important role in supporting MM cell growth.

Yaccoby and coworkers have shown that primary MM plasma cells from patients are attracted to OCL precursors and that MM cells induce differentiation of these cells into multinucleated bone resorbing OCL [4]. They further showed that a coculture of MM cells with OCL allowed the primary MM cells to proliferate for more than 13 weeks. Physical contact between OCL and MM cells was required for these effects, and both OCL from healthy donors and MM patients could support the growth of MM cells. Blocking IL-6 decreased survival of MM cells but had no effect on the proliferation of the primary MM cells. Similarly, Abe and co-workers [5] have shown that OCL support the growth of primary MM cells and that this is dependent on both osteopontin and IL-6 production by the OCL. These authors demonstrated that peripheral blood mononuclear cell-derived OCL were much more potent in enhancing the growth and survival of primary MM cells than BMSC. They also showed that OCL protected MM cells from apoptosis induced by serum depletion or treatment of MM cells with doxorubicin. Again, adhesion of the MM cells to

OCL was required to support MM cell growth as complete inhibition of cell contact between MM cells and OCL totally blocked the supportive effects of OCL on MM cell growth. These data clearly showed that OCL play a pivotal role in the support of MM cell growth. The adhesive interactions between MM cells and OCL increased IL-6 production by OCL. Osteopontin (Opn) receptors, VLA-4, $\alpha_v\beta_3$-integrin, and CD44 are expressed on the cell surface of myeloma cells. IL-6 and Opn in combination enhanced MM cell growth and survival. However, other factors must also be involved in OCL supported MM cell growth, because it is only partially inhibited by simultaneous addition of anti-osteopontin and anti-IL-6 antibodies. As discussed further below, IL-6 has multiple sources and roles in MM bone disease; however, IL-6 production by OCLs may increase MM tumor burden leading to enhanced bone destruction.

Other OCL-derived factors have been implicated in the support of myeloma cells. Ge et al. found that the DASH protease, fibroblast activation protein (FAP), was involved in the OCL-induced MM cell growth [6]. These authors demonstrated that FAP was upregulated when OCL and MM cells were cocultured in vitro as well as in MM tissue in human bone in the SCID-hu model of MM. FAP was expressed by OCL and was critical for the support of MM cell growth by OCL. In addition, knockdown of FAP expression with a siRNA reduced MM cell survival in these cocultures. Inhibition of DASH proteases with PT-100 affected expression of adhesive molecules by OCL that are required for OCL support of MM cell growth and MM bone disease [7]. Further, inhibition of DASH proteases blocked OCL differentiation and bone resorption activity. Tanaka and coworkers [8] have shown that MM cell–OCL interactions enhance angiogenesis. These authors found that OCL-derived osteopontin and VEGF produced by MM cells cooperatively enhanced angiogenesis and induced osteoclastogenic activity by vascular endothelial cells. These data clearly show that the OCL plays a central role in both MM cell growth and the increased angiogenesis associated with MM. Further, Abe and coworkers reported that BAFF and APRIL are OCL-derived survival factors for MM cells [9], which are also produced by bone BMSC from myeloma patients. Thus, BAFF, produced by both OCL and BMSC in patients with MM, is a potential therapeutic target for treating MM bone disease.

Recently, we have found that OCL produce annexin II (AXII), which is a stimulator of MM cell growth [10], by both increasing proliferation and decreasing apoptosis [11] and is also an autocrine/paracrine stimulator of OCL formation [12, 13]. AXII was found to be upregulated in pancreatic, stomach, lung, renal, breast cancers, and more importantly in MM [14–20]. More recently, AXII was shown to increase the proliferation of human MM cell lines and had anti-apoptotic effects in these MM cell lines [11]. The AXII/AXII receptor (AXIIR) axis plays a crucial role in the homing, growth, and adhesion of prostate cancer cells to the bone marrow [21]. AXII appears to stimulate MM cell growth through increased ERK and p38 MAPK signaling. This is consistent with previous studies in which we have shown that AXII can also stimulate receptor activator of NF-κB ligand (RANKL) expression in human BMSC via MAPK as well as GM-CSF expression by both marrow stromal cells and activated T cells [10, 22]. RANKL and GM-CSF together are important

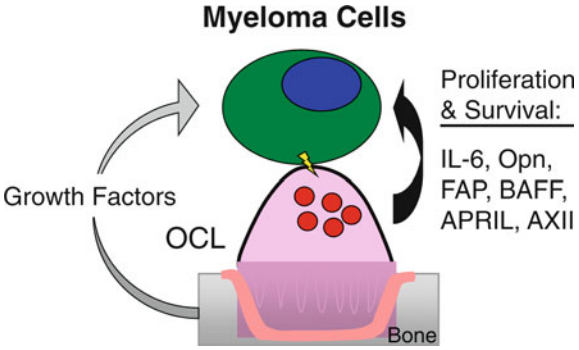

Fig. 9.1 *Mechanisms of osteoclastic support for myeloma cells*. Osteoclasts are stimulated by cell–cell contact with myeloma cells to produce a variety of factors that support myeloma proliferation and survival, such as IL-6, Opn, FAP, BAFF, APRIL, and AXII. The bone destructive process releases growth factors that increase the growth of myeloma cells and increase OCL progenitors, further exacerbating both processes

for OCL formation induced by AXII. MM cells themselves also make AXII, but it appears in preliminary studies that MM-derived AXII does not increase MM cell growth, whereas both OCL- and BMSC-derived AXII stimulate the growth of MM cells. Thus taken together, these data demonstrate a critical role for OCL in the support of MM cell tumor proliferation and prevention of MM cell apoptosis (Fig. 9.1).

9.3 Osteoclast Stimulatory Factors Produced in Myeloma

In addition to factors produced by OCLs, osteoclastic bone resorption releases growth factors, which enhance the growth of MM cells (Fig. 9.1). This has been termed the "vicious cycle" for MM cell growth in which MM cells induce increased OCL activity and the bone resorption process releases immobilized growth factors produced by the marrow microenvironment that both support the MM cells and further stimulate OCL. Locally acting factors produced by MM cells have been implicated in both the extensive bone destruction and impaired new bone formation. The factors produced in vivo by MM cells or induced by MM in bone microenvironmental cells that can increase osteoclastic activity include RANKL, macrophage inflammatory protein-1α (MIP-1α), TNF-α, IL-1β, IL-3, and IL-6 [23–27] (Fig. 9.2).

RANKL is part of the tumor necrosis factor (TNF) gene family and is a major osteoclastogenic factor involved in MM bone disease. When MM cells bind to BMSC, RANKL expression is increased on the surface of the BMSC. Subsequently, this results in enhanced OCL activity through binding of RANKL to its receptor RANK on OCL precursor cells, promoting their differentiation [28]. RANKL also plays a role in the inhibition of OCL apoptosis [29]. T-lymphocytes also produce RANKL in the MM marrow microenvironment. The proposed mechanism for the

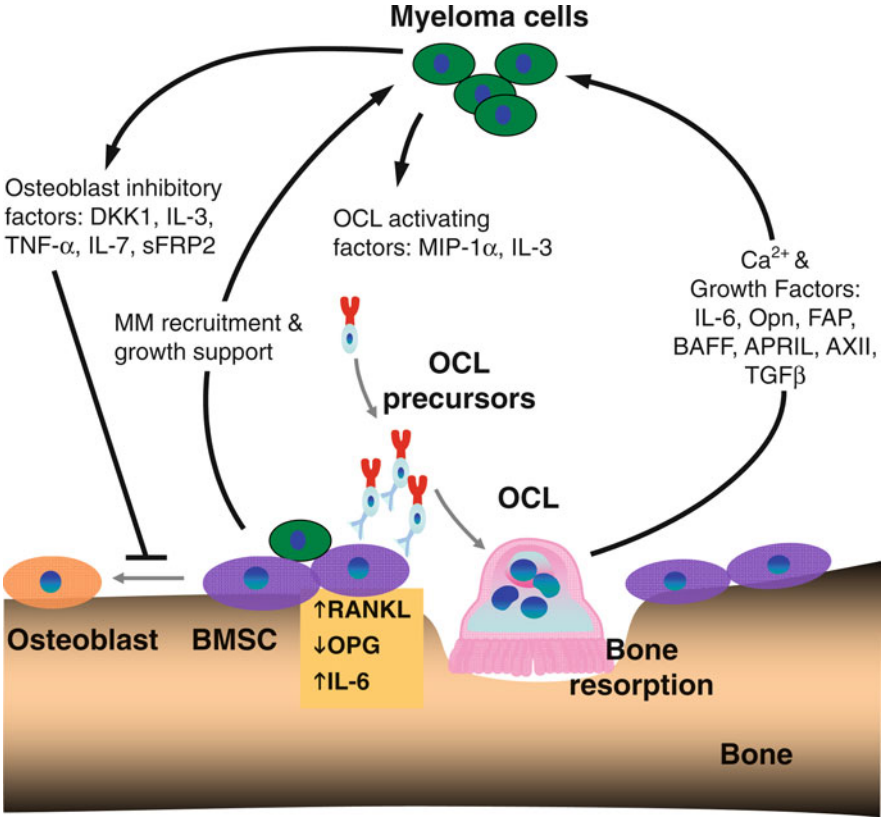

Fig. 9.2 *Mechanisms responsible for myeloma bone disease*. Myeloma cells produce factors that directly or indirectly activate osteoclasts such as MIP-1α, TNF-α, IL-1β, and IL-3. In addition, MM cells enhance osteoclast formation and activation by inducing BMSC production of IL-6 and altering the RANKL/OPG ratio. Myeloma cells also produce dickkopf-1 (DKK-1), IL-3, soluble frizzle-related protein-2 (sFRP2), TNF-α, and IL-7, which suppress osteoblast differentiation and new bone formation

upregulation is through the release of a soluble factor by MM cells, which increases RANKL expression on the T-lymphocytes and BMSC and ultimately results in enhanced osteolytic bone destruction [30].

A soluble decoy for RANKL, known as osteoprotegerin (OPG), is produced by BMSC and inhibits the actions of RANKL on osteoclastogenesis. The ratio of RANKL to OPG determines the level of OCL formation and activity. Interactions between MM cells and BMSC lead to decreased production of OPG, which allows for increased amounts of RANKL binding to its receptor. This results in further OCL activation and enhanced bone destruction [29]. Giuliani et al. have demonstrated that in cocultures of human MM cells with BMSC, RANKL expression was upregulated and OPG production strongly downregulated at both the protein and mRNA levels in the BMSC [25]. In addition, Pearse et al. have examined bone marrow

biopsy specimens from patients with MM and found that RANKL expression was markedly upregulated in bone marrow biopsies from patients with MM, while OPG was expressed at very low levels compared to normal controls [31]. The above studies suggest that there is a marked imbalance between RANKL expression and OPG levels that favors osteoclastogenesis and OCL activation in MM.

In a murine model of MM, Menu et al. demonstrated that injected Fc-OPG inhibited the development of MM-induced osteolytic bone disease and also led to a significant reduction in tumor load [32]. Similarly, when primary MM cells are injected into a human fetal bone rudiment implanted into mice with severe combined immunodeficiency (SCID), a RANKL inhibitor, RANK-Fc, decreased bone resorption and tumor burden [33]. These studies suggest that blocking bone resorption induced by RANKL may decrease tumor burden as well as bone destruction in patients with MM. Based on these observations, a human monoclonal antibody to RANKL has been developed and used in phase I, II, and III trials in MM patients, and is discussed below.

Recently, antagonists to the MIP-1α receptor, CCR1, have been developed, and tested in vitro and in vivo in preclinical models. These experiments have demonstrated their potential utility in treating MM bone disease. Oba and coworkers reported that the CCR1 antagonist, BX471, inhibited OCL formation induced by MIP-1α and blocked adhesion of MM cells to BMSC. This resulted in decreased secretion of IL-6 by the BMSC [34]. Similarly, Vallet et al., using another CCR1 antagonist MLN3897, showed that MLN3897 inhibited OCL formation and inhibited the adhesion of MM cells to OCL, thereby decreasing MM cell growth and survival [35]. Menu et al. have reported studies using the 5TMM mouse model of MM in which BX471 decreased development of osteolytic lesions by 40% in mice with established tumors [36]. Taken together, these results demonstrate that CCR1 is a viable target for treating MM bone disease and should be pursued. It is expected that CCR1 antagonists will be in clinical trial for MM in the next several years.

TNF-α and IL-1β induce IL-6 and RANKL production [37] and can also synergize with RANKL to potentiate OCL formation (TNF-α) [38] as well as OCL activation and survival (IL-1β) [39]. However, their source and roles in MM bone disease are unclear [23, 40]. In particular, a pilot study of recombinant human soluble TNF receptor fusion protein (Etanercept) in patients with refractory multiple myeloma did not result in an objective response. Furthermore, acceleration of disease occurred in four of ten patients [41]. In a phase II clinical trial with 47 patients with smoldering and indolent MM who were at risk of progression to active myeloma, treatment with IL-1 receptor antagonist (IL-1Ra) and low-dose dexamethasone was reported at ASCO 2007 to induce a chronic disease state with improved progression-free survival [42]. More recently, in preclinical studies, a humanized anti-IL-1β antibody (XOMA 052) was highly effective at inhibiting IL-6 production generated by all MM patient supernatants from bone marrow cells tested including the patients that were high inducers of paracrine IL-6 production.

IL-3 is also significantly elevated in marrow plasma from patients with MM as compared to normal controls [26]. Previous reports have shown that up to 40% of patients with MM will have elevated levels of IL-3 in the peripheral blood, and 75%

of bone marrow samples from patients with MM will have elevated IL-3 mRNA and protein levels [24]. Serum from MM patients with elevated IL-3 stimulates the growth of IL-3 dependent MM cell lines [43]. IL-3 can induce in vitro OCL formation in human marrow cultures at levels similar to those measured in MM patient samples, and OCL formation induced by marrow plasma from MM patients could be inhibited by a blocking antibody to IL-3 [26]. IL-3 also enhances the effects of RANKL and MIP-1α on the growth and development of OCLs, as well as directly stimulates MM cell growth [26]. Further, addition of IL-3 to murine bone marrow induces the development of OCL-like cells, which were multinucleated and stained positively for tartrate resistant acid-phosphatase (a marker enzyme of OCLs) [44]. Overall, IL-3 increases the numbers and activity of OCLs, leading to further bone destruction, and appears to be an OCL stimulatory factor in MM.

The role that IL-6 plays in MM is controversial. It is unclear if elevated levels of IL-6 correlate with disease status [24, 45]. Levels of IL-6 have been shown to be elevated in patients with osteolytic lesions, as compared to patients without lytic lesions or with patients with monoclonal gammopathy of undetermined significance (MGUS) [46]. IL-6 levels from bone marrow, but not peripheral blood plasma, have also been correlated with markers of bone turnover [47]. IL-6 induces RANKL expression in mesenchymal cells thereby increasing osteoclastogenesis [48, 49]. Most studies support the idea that IL-6 is produced by cells in the bone marrow microenvironment induced through contact with MM cells. These cell types include osteoblasts, OCLs, and BMSC. Increased osteoblast production of IL-6 has been reported in cocultures of human osteoblasts with MM cells [50]. OCLs also produce high levels of IL-6 when grown in coculture with MM cells [5]. The increased IL-6 not only increases OCL formation but also further enhances the growth of the MM cells and inhibits MM cell apoptosis [5, 51]. Based on these observations, humanized monoclonal antibodies to both IL-6 and IL-6R have been developed and will be discussed below.

Because multiple signaling pathways are activated in BMSC from MM patients, that regulate both BMSC support of MM and induction of osteoclast formation, attempts have been made to try to identify a common component that is involved in these multiple signaling pathways and can be targeted to treat MM bone disease. BMSC from MM patients have increased NF-κB and p38 MAPK signaling. p62 is a common component that serves as a platform for formation of these signaling complexes. However, the effects of targeting p62 on these signaling pathways in MM are unknown. We found that although p62 levels were not altered in the BMSC of 13 MM patients compared to 11 healthy controls, signaling through p62 was increased in BMSC from MM patients compared with healthy cells as exemplified by elevated ratios of phosho-PKCζ to total PKCζ (two to sixfold), although the levels varied greatly among the individual patients. Therefore, we determined the effects of siRNA knockdown of p62 in BMSC on p38 MAPK and NF-κB signaling. p62 expression was decreased by 60% and 90% at the mRNA and protein level, respectively, in these BMSC. PKC and VCAM-1 expressions were decreased by at least 70% in p62 siRNA transduced MM-derived and normal BMSC compared with control siRNA transduced cells. Further, knocking-down p62 in primary MM-derived

BMSC treated with TNF-α markedly decreased NF-κB and p38 MAPK signaling compared with control siRNA treated cells. Importantly, IL-6 production by p62 siRNA transfected normal and MM-derived BMSC was also significantly decreased compared with scrambled siRNA or untreated cells. We further showed that loss of p62 markedly decreased the capacity of MM patient-derived BMSC to both induce OCL formation and enhance the growth of MM cells. These results demonstrate that targeting p62 may be a method for blocking the role of the microenvironment in MM bone disease.

9.4 Targeting Osteoclast Generation and Activity to Inhibit Tumor Growth in Myeloma

9.4.1 Bisphosphonates

Nitrogen-containing bisphosphonates interfere with OCL function and survival and have been extensively utilized to treat osteoporosis [52]. These compounds bid avidly to the surface of bone hydroxyapatite crystals and are ingested by OCL during bone resorption. These drugs interfere with metabolic pathways involving diphosphate moieties such as the mevalonate pathway involved in cholesterol synthesis and prenylation of GTPases Rab, Rho, and Ras. This leads to disturbance of the OCL cytoskeleton resulting in decreased bone resorption and increased OCL apoptosis.

9.5 Anti-Myeloma Effects of Bisphosphonates in Preclinical Models of Myeloma

Studies in preclinical models of MM and bone metastases [53–56] demonstrated that bisphosphonates inhibit tumor growth and decrease bone destruction in vivo. Yaccoby and coworkers reported that pamidronate and zoledronate decreased tumor growth in a SCID-hu model of MM [57]. In this model, human fetal bone is implanted subcutaneously in mice with severe combined immunodeficiency. Primary human MM cells are then injected into the fetal bone. The MM cells grow in this human microenvironment and induce bone resorption. Treatment of these mice with pamidronate or zoledronate inhibited MM-induced bone resorption and MM cell growth, if MM cells were from patients with disease confined to the bone marrow. In contrast, pamidronate and zoledronate did not inhibit tumor growth when MM cells from patients with extramedullary disease were used. These results suggested that the anti-MM effects of bisphosphonates only occurred if the MM cells were dependent on the marrow microenvironment and/or bone resorption for growth.

Similarly, Croucher et al. used the 5T2MM model of MM to test the effects of bisphosphonates on MM growth and bone destruction [58]. The 5T2MM model of MM is an immunocompetent model of MM in which murine MM cells derived from a spontaneously developing MM in mice are injected intravenously into syngeneic hosts. The mice develop a disease that has all the characteristics of human MM. Zoledronate treatment, either from time of tumor injection or after paraprotein was detected, prevented osteolytic lesions, decreased tumor burden, and significantly increased survival of the mice from 35 to 47 days after detection of the paraprotein. Zoledronate also blocked the increased angiogenesis induced by the MM cells. These results suggest that bisphosphonates inhibit tumor-induced angiogenesis through their effects on MM cells and/or on endothelial cells. Radl et al. [59] reported that pamidronate also reduced tumor burden and increased survival in the 5TMM2 model of MM.

However, bisphosphonates also significantly reduce the growth of prostate, lung, and breast cancer cells implanted subcutaneously in mice (reviewed in [60]), suggesting that bisphosphonates can also inhibit tumor growth independent of their effects on bone remodeling. Bisphosphonates can also directly inhibit growth, induce apoptosis, and increase sensitivity to chemotherapy in MM cell lines. Guenther and coworkers [56] reported that zoledronate inhibited the growth of six different MM cell lines. Importantly, the concentrations of zoledronate required to induce cytotoxicity in MM cells did not affect peripheral blood mononuclear cells from healthy donors. Baulch-Brown and coworkers also showed that zoledronate inhibited MM cell growth and that the inhibitory effects of zoledronate on MM cell growth were due to its capacity to prevent geranylgeranylation of small GTPases that resulted in cell cycle arrest and apoptosis [61]. Bisphosphonates also inhibit MM cell adhesion to BMSC [62], increasing the sensitivity of MM cells to chemotherapy [63]. Since small GTPases play a key role in integrin activation, the inhibition of tumor cell adhesion to matrix or BMSC by bisphosphonates is not surprising [64]. Further, zoledronate inhibits chemokine-induced tumor cell migration by affecting cell surface expression of the chemokine receptor CXCR4, a receptor for CXCL12 [65]. CXCR4 and CXCL12 play important roles in MM cell homing to the marrow and MM cell mobilization to the peripheral blood [66]. Finally, zoledronate can synergize with several chemotherapeutic agents, to increase tumor cell apoptosis and enhance TNF-α related apoptosis through TRAIL [66, 67]. These in vitro results demonstrate the direct anti-MM potential of bisphosphonates.

9.6 Clinical Studies Reporting Effects of Bisphosphonates in Treatment of Myeloma

The seminal studies of Berenson and coworkers [68] demonstrated that pamidronate significantly increased the time to development and decreased the number of skeletal related events (SREs) as well as bone pain in patients with advanced MM. However, pamidronate did not significantly increase survival of these patients.

Attal and coworkers examined the efficacy of pamidronate as maintenance therapy for MM patients after autologous stem cell transplantation [69]. Six hundred patients were randomly assigned to receive no maintenance, pamidronate, or pamidronate with thalidomide following autologous stem cell transplantation. None of the patients received pamidronate prior to transplantation. Pamidronate did not decrease SREs or increase 3-year event-free or overall survival in the patients. In contrast, both event-free survival and overall survival were significantly increased in patients receiving pamidronate with thalidomide. These results demonstrated that pamidronate as a single agent did not confer a survival advantage in patients with MM. However, this trial could not distinguish if pamidronate enhanced the effects of thalidomide on event-free and overall survival because no patients received thalidomide without pamidronate in the trial agent. Anecdotally, Kondo and coworkers reported an MM patient treated for 18 months with pamidronate and no additional anti-MM treatment [70]. Pamidronate markedly reduced marrow plasmacytosis and sbin levels in this patient.

Several studies have shown that bisphosphonates have antitumor effects in breast cancer patients when used in the adjuvant setting. Diel and coworkers and Powles et al. reported that treatment of patients with primary breast cancer at high risk for distant metastasis with clodronate decreased bone metastasis and increased overall survival compared to placebo [71, 72]. Visceral metastases also decreased in patients treated with adjuvant clodronate [73]. Gnant et al. recently reported that treatment of premenopausal breast cancer patients with endocrine therapy and zoledronate improved disease-free survival as well as decreased bone and distant metastasis but did not improve overall survival [74]. Further, large trials of zoledronate for prevention of treatment-induced bone loss in premenopausal breast cancer patients receiving aromatase inhibitors or postmenopausal patients receiving adjuvant endocrine therapy for stages 1 to 3A hormone responsive breast cancer found a significant decrease in both bone and distant metastasis as well as increased disease-free survival (reviewed in [75]). In addition, patients receiving neoadjuvant chemotherapy and zoledronate had an increased complete remission rate as well as decreased residual tumor size at surgery [75]. These results suggest that zoledronate may have antitumor effects in breast cancer patients independent of its effects on bone.

Until recently, a distinct survival advantage for zoledronate treatment of patients with MM has not been reported [76]. Avilés et al. treated 94 newly diagnosed MM patients with conventional chemotherapy and either zoledronate or placebo [77]. Five-year actuarial event-free survival and overall survival was increased for patients receiving zoledronate compared to controls (80% vs. 46%, $p<0.01$). However, this trial did not determine if the effects of zoledronate on survival were independent of zoledronate's effects on SREs. However, at the 2010 American Society of Clinical Oncology Meeting, Morgan and colleagues reported the results of the MRC Myeloma IX trial [78]. This was a prospective multicenter randomized controlled trial comparing intravenous zoledronate (4 mg every 3–4 weeks) with daily oral clodronate in patients randomized to either intensive therapy, which included stem cell transplantation, or less intensive therapy. MM treatment was

dependant on the performance status of the patient. Almost 2,000 newly diagnosed MM patients were entered into this trial. Patients had international staging system (ISS) stage I, II, or III MM. Approximately 20% of the patients did not have bone disease. At a median follow-up of 3.7 years, SREs were significantly reduced in patients treated with zoledronate as compared to clodronate (27% vs. 35%, $p=0.0004$). Importantly, patients treated with zoledronate had a 5.5-month survival advantage compared to those receiving clodronate. Zoledronate treatment decreased the risk of death by 16% and progress-free survival by 12% ($p=0.0118$ and $p=0.0179$, respectively). Multivariate analysis demonstrated that this survival advantage was independent of zoledronate's effects on SREs. The incidence of osteonecrosis of the jaw was low in the study (3.6% vs. 0.3% in zoledronate vs. clodronate-treated patients). Further, Dr. Morgan stated at the presentation that patients who do not have bone disease and received zoledronate also had a similar survival advantage compared to clodronate.

How zoledronate enhanced the survival of MM patients in this large prospective randomized trial is unclear. Zoledronate could affect patient survival through its effects on OCL, or it may have direct effects on MM cells. OCL are angiogenic cells [79], and zoledronate's inhibition of OCL activity may contribute to decreased angiogenesis in MM patients. Another potential mechanism for the enhanced disease-free survival of MM patients receiving zoledronate could be prevention of MM cell mobilization to distant bone marrow sites. Kollet and colleagues reported that OCL play a role in hematopoietic stem cell mobilization through degradation of CXCL12 [80]. The CXCR4/CXCL12 axis also contributes to mobilization of MM cells from the bone marrow of patients with MM [81]. Thus, blocking OCL activity should inhibit MM cell mobilization. However, patients without bone disease had the same survival advantage as those with bone disease, and multivariate analysis found that the survival advantage was independent of SREs. Nitrogen-containing bisphosphonates can have immunomodulatory effects and stimulate expansion of γΔ-T cells, thereby increasing tumor cell lysis by γΔ-T cells [82]. Zoledronate could also affect tumor growth through its effects on endothelial cells, angiogenesis, and decreasing VEGF production, as demonstrated in patients with metastatic breast cancer [78]. Thus, the effects of zoledronate on patient survival cannot be completely explained by its inhibition of OCL activity. However, because of the complexity of this trial, further analysis will be required to determine the mechanism(s) responsible for the survival advantage conferred by zoledronate in MM patients.

9.6.1 RANKL Inhibition as a Target to Inhibit Tumor Growth in Myeloma

Preclinical and clinical studies clearly identified the importance of RANKL as a driver of osteoclastogenesis in MM, and several studies have suggested that MM cells themselves can produce RANKL as well as induce BMSC and activated T cell

RANKL production [83, 84]. Importantly, preclinical studies using OPG have shown that blocking RANKL activity markedly decreases bone destruction and tumor burden in murine models of MM [85, 86]. These studies have led to the development of a high infinity human monoclonal antibody that binds RANKL, denosumab. Denosumab specifically binds RANKL and does not bind other gene family members such as TNF-α, TNF-β, TRAIL, or CD40 [87]. It directly inhibits OCL formation and activation as well as affects OCL survival. Phase I studies have shown that denosumab at 1–3 mg/kg given subcutaneously as a single dose can suppress bone resorption markers for up to 90 days. The suppression of bone resorption markers induced by denosumab was at the same level as that seen with a single dose of 90 mg of pamidronate. However, pamidronate suppression of bone resorption markers only lasted about 30 days [88]. A phase II study of denosumab in patients with relapsed and plateau phase MM showed that denosumab was very effective for MM bone disease with bone resorption markers decreased in relapsed patients by 70% and a 52% decrease in bone resorption markers in plateau phase patients [89]. Recently, results of a phase III trial that compared denosumab to zoledronic acid in MM in patients with solid tumor bone metastasis, but not breast cancer or prostate cancer, have been reported [90]. Denosumab was noninferior in delaying or preventing the first on study skeletal-related event compared to zoledronic acid in over 1,600 patients, of which approximately 200 were MM patients. Further, adverse event rates with denosumab and zoledronic acid were similar, and the incidence of ONJ was infrequent and not significantly different between the treatment arms (10 vs. 11 patients). Thus, denosumab is equally efficacious as zoledronic acid in patients with MM although it is unclear what the long-term effects of denosumab will be because of the small number of MM patients in the phase III study.

9.6.2 Blocking IL-6 to Treat Myeloma Bone Disease

Both IL-6 and IL-6R (gp80) have been targeted through the development of humanized mAbs (reviewed in [91, 92]). Anti-IL-6 antibodies developed by Diaclone (B-E8) and Centocor (CNTO 328) have been used alone or in combination with chemotherapeutic agents in preclinical studies and in small phase I clinical studies of MM. Both B-E8 (half-life 3–4 days) and CNTO 328 (half-life 18 days) transiently blocked IL-6 action, decreased C-reactive protein production, generated antiproliferative effects, and decreased IL-6 toxic effects such as fever and were well tolerated. It is not clear why the therapeutic effects of both anti-IL-6 antibodies were transient. The anti-IL6R mAb (Tocilizumab/Actemra®) is already in use for treatment of Castleman's disease and rheumatoid arthritis (specifically blocking inflammatory osteoclastogenesis) and has shown effectiveness for juvenile idiopathic arthritis and Crohn's disease. However, phase I/II clinical trials in MM have just begun.

9.7 Summary

The OCL appears to play a critical role in supporting the growth of MM cells, both by the direct effects of factors produced by OCL, including BAFF, APRIL, osteopontin, IL-6, and AXII. In addition, the bone destructive process ongoing in MM cells releases growth factors, which stimulate the growth of MM cells from the bone microenvironment. Targeting OCL activity in MM with bisphosphonates appears to improve survival of MM patients and suggests that combinations of therapies that target both OCL activity and the tumor cells themselves should have a profound effect on MM bone disease and MM tumor growth in general. Future studies with denosumab will determine if it too has anti-MM effects comparable to those recently reported with zoledronic acid.

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