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REVIEW

Effects of proteasome inhibitors on bone cancer

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Bone metastasis is a frequent complication of cancer, occurring in up to 70% of patients with advanced breast or prostate cancer, while bone disease is also the characteristic clinical feature of multiple myeloma. Skeletal-related events can be devastating, with major effect on the quality of life and survival. Bisphosphonates are the mainstay of therapeutic management of bone disease of solid tumors and myeloma, and denosumab has recently been approved for patients with bone metastases. Both act through inhibition of the osteoclast activity but do not restore bone formation. Proteasome inhibition has direct bone anabolic effects. Proteasome inhibitors have been used in the management of patients with multiple myeloma and mantle-cell lymphoma during the last decade. In multiple myeloma, bortezomib, the first-in-class proteasome inhibitor, has shown both *in vitro* and *in vivo* regulation of bone remodeling by inhibiting osteoclast function and promoting osteoblast activity. Bortezomib also reduces bone resorption but more importantly increases bone formation and bone mineral density, at least, in subsets of myeloma patients. Thus, bortezomib is recommended for myeloma patients with extended bone disease in combination with bisphosphonates. This review focuses on the effects of the proteasome system on bone metabolism and the implications into the better management of patients with cancer and bone disease.

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Introduction

Bone metastasis is a frequent complication of cancer, occurring in up to 70% of patients with advanced breast or prostate cancer.[1] Bone disease is also the hallmark of multiple myeloma (MM), present in 80% of the MM patients, characterized by the presence of lytic lesions and/or osteoporosis.[2] Skeletal-related events involving pathological fractures, spinal cord compression and a need for surgery/radiotherapy, which are frequently observed in cancer patients with bone metastases and myeloma, can be devastating, with major effect on quality of life and survival.[3] Bisphosphonates are the cornerstone of therapeutic management of bone disease of solid tumors and myeloma, offering considerable benefit in preventing or delaying skeletal-related events and relieving pain, whereas denosumab has been approved for patients with bone metastases from solid tumors.[1,3] However, these agents have an impact on osteoclast activity only and do not restore bone formation. Thus, new approaches to inhibiting osteoclastic resorption and preventing the inhibition of bone formation are still required to prevent the development of bone disease. This review focuses on the effects of the inhibition of the proteasome system, which seems to have a key role in regulating bone remodeling by inhibiting osteoclast formation and stimulating new bone formation, on bone cancer.

Mechanisms of Cancer-Related Bone Disease

The pathophysiology of myeloma bone disease has been studied extensively over recent years, leading to new insights into the complex interactions between myeloma cells, osteoclasts and osteoblasts (**Figure 1**).[3] Histomorphometric studies have revealed that myeloma cells promote osteoclastic bone resorption and suppress osteoblast activity.[4] The bone destruction brought about by the myeloma cells results in the release of cytokines and growth factors, either from osteoclasts themselves or from the bone matrix, which may further promote myeloma cell growth and survival, creating a vicious cycle of tumor expansion and bone destruction.[5] The biologic pathway of the receptor activator of nuclear factor-kappa B (RANK), its ligand (RANKL) and osteoprotegerin (OPG), which is the decoy receptor of RANKL, is of major importance for the increased osteoclast activity observed in MM. Myeloma cells disrupt the balance between RANKL and OPG by increasing the expression of RANKL and decreasing the expression of OPG. The resulting increase in RANKL favors the formation and activation of osteoclasts, leading to increased bone resorption.[6,7] More recently, activin-A has been implicated in MM bone disease, through the stimulation of RANK expression and inducing osteoclastogenesis.[8,9] On the other hand, in addition to their stimulatory effect on osteoclasts, myeloma cells have

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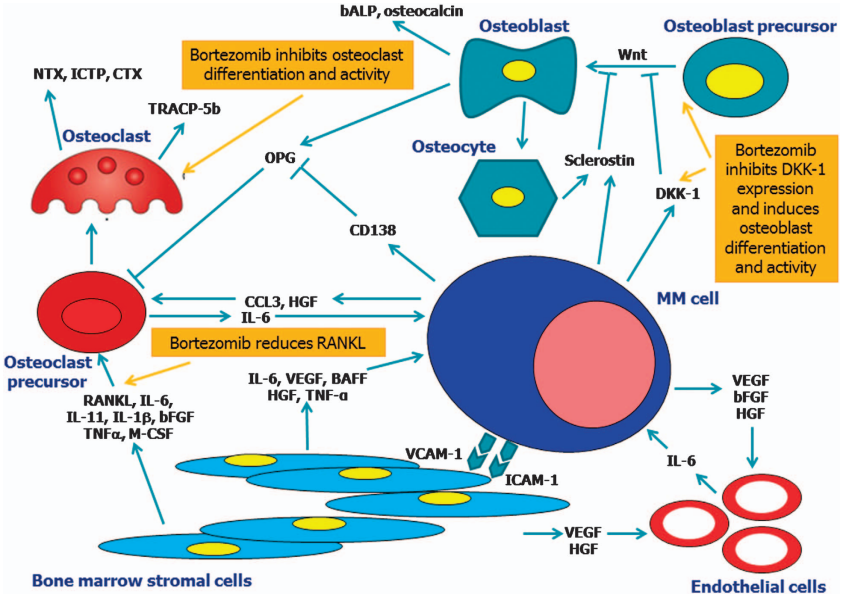

Figure 1 Pathophysiology of myeloma bone disease and the possible sites of action of bortezomib. Myeloma cells adhere to bone marrow stromal cells (BMSCs) through the binding of very late antigen-4 and lymphocyte function-associated antigen-1 (present on the surface of MM cells) to vascular cell adhesion molecule-1 and intercellular adhesion molecule-1, respectively, which are expressed on stromal cells. The adherence of MM cells to the BMSCs enhances the production of the receptor activator of nuclear factor-kappa B ligand (RANKL) and other cytokines with osteoclast activating function activity (interleukin (IL)-6, IL-11, interleukin-1β, tumor necrosis factors, basic fibroblast growth factor (bFGF), whereas it suppresses the production of OPG (the decoy receptor of RANKL). Furthermore, myeloma cells produce chemokine C-C motif ligand-3, hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF), which enhance the proliferation and differentiation of osteoclast precursors. Myeloma cells may express RANKL, while OPG binds both surface and soluble RANKL, inhibiting osteoclast development and bone resorption. Syndecan 1 (CD138) expressed on the surface of, and secreted from, the myeloma cells can bind soluble OPG, thus preventing its inhibitory effect on RANKL function. Therefore the ratio of RANKL/OPG is increased, leading to osteoclast differentiation, proliferation and activation, and increased bone resorption, as is reflected by the increased levels of bone resorption markers (TRACP-5b, NTX, ICTP, CTX). Bortezomib restores the RANKL/OPG balance and inhibits osteoclast differentiation and activity. The production of VEGF, bFGF and HGF by MM cells and BMSCs results in increased angiogenesis. In turn, IL-6 produced by osteoclasts, endothelial cells and BMSCs increases the growth of MM cells. On the other hand, Wnt signaling inhibitors, such as Dkk-1 and sclerostin, produced by MM cells and osteocytes in apoptosis, respectively, disrupt osteoblast maturation and function, with reduced formation of new bone, reflected by decreased levels of bone formation markers (bALP, OC). The net result of all these complex interactions is tumor expansion and ostelolytic bone disease. Bortezomib inhibits Dkk-1 expression, increases osteoblast differentiation and activity, and leads to increased bone formation.

been shown to suppress bone formation.[3] The Wingless-type (Wnt) signaling pathway has been shown to have a key role in osteoblast differentiation and has been implicated in osteoblast suppression in myeloma. The Wnt signaling inhibitors dickkopf-1 (Dkk-1) and sclerostin are secreted by myeloma cells and osteocytes, respectively, and have been found to be increased in the serum of myeloma patients, leading to the blockage of osteoblast differentiation and activity.[10–13] Soluble frizzle-related protein-2, another inhibitor of Wnt signaling, has also been implicated in the suppression of bone formation in myeloma.[14] Although the circulating levels of the above molecules and mainly of sclerostin have not been found to be elevated in myeloma patients in all published studies, the importance of Wnt inhibition in the biology of myeloma-related bone disease is undoubted. These differences support the notion that the biological phenomena that take place in the microenvironment are not always reflected in the periphery.

In the field of bone metastasis of solid tumors, the bone marrow microenvironment has also been established as a regulatory site for localization and survival of tumor cells.[15] Osteoblasts in particular may play a critical regulatory role, expressing a milieu of secretory factors and adhesion molecules that have been shown to be important for solid tumor metastasis to the bone marrow.[16]

The role of RANKL/OPG system has also been studied in patients with solid tumors metastatic to the bone in relation to the type of malignancy and the neoplastic burden to the skeleton. Breast and lung cancer seem to exert their osteolytic action through upregulation of the RANKL/OPG system, whereas prostate cancer seems to provoke profound elevation of OPG levels only, thus leading to increased osteoblastic activity.[17] The differential expression of RANKL, RANK and OPG has been associated with the metastatic potential of human non-small cell lung cancer and human prostate cancer to skeleton.[18,19] Inhibition of RANKL blocks skeletal tumor progression in mouse models of breast and prostate cancer bone metastasis.[20,21] The Wnt signaling pathway is also implicated in solid tumors. Dkk-1 expression is increased in breast cancer bone metastases and decreased in metastatic prostate cancer.[22,23]

The Ubiquitin–Proteasome Pathway and Bone Metabolism

The proteasome, a multi-catalytic enzyme complex, is the main intracellular, extra-lysosomal, proteolytic system involved in intracellular proteolysis. It is highly selective with regulated actions that ensure the rapid degradation of target proteins through the ligation of chains of ubiquitin. In addition to regulating protein homeostasis, the ubiquitin–proteasome pathway is an important mechanism for the regulation of cell cycle, transcriptional activation, apoptosis and cell signaling stimuli. If the degradation of proteins such as cell cycle regulators is disturbed, the impact may be greatest in neoplastic cells, because their faster proliferative rate and defective

cell cycle checkpoints make them more vulnerable to pro-apoptotic stimuli compared with normal cells. Thus the ubiquitin–proteasome pathway is directly related to the development and progression of cancer, and may also be directly involved in regulating bone turnover.[24]

Proteasome inhibition is implicated in the regulation of bone metabolism through the reduction of RANKL-mediated osteoclast differentiation via the inhibition of nuclear factor-kappa B (NFκB) signaling. Binding of RANKL to RANK on the surface of osteoclast precursors induces NFκB activation, which leads to osteoclast differentiation and bone resorption. However, in the presence of proteasome inhibitors, IκB, the inhibitor of NFκB, which is normally degraded by the proteasome, remains bound to NFκB and prevents the activation of NFκB.[25] In a German study, treatment of osteoclast precursors with the proteasome inhibitors MG-132 and MG-262 inhibited RANKL-mediated osteoclast formation, accompanied by a reduction of the resorption capacity of osteoclasts. The diminished osteoclast differentiation was strongly correlated with the reduction of NFκB activation, which was dose-dependent. Bortezomib also inhibits human osteoclast differentiation, activation and resorptional activity in a dose- and time-dependent manner.[26]

On the other hand, the ubiquitin–proteasome pathway is also involved in the regulation of osteoblast differentiation and bone formation. Proteasome inhibitors, such as epoxomicin, proteasome inhibitor-1 and lactacystin, were shown to promote bone formation and increase osteoblast numbers in a dose-dependent manner. The effect of proteasome inhibitors on bone formation was reported to be mediated by increased bone morphogenetic protein (BMP)-2 expression in osteoblasts. BMPs act predominantly on osteoblasts and promote their differentiation in an autocrine manner.[27] These data strongly suggest that the proteasome system may have a key role in regulating bone remodeling by inhibiting osteoclast formation and stimulating new bone formation.

Proteasome Inhibition and Cancer-Related Bone Disease

Bortezomib is the first-in-class potent and reversible inhibitor of the proteasome that has demonstrated efficacy in the treatment of both MM and lymphomas.[28,29] Bortezomib has been approved for the treatment of MM patients who have received at least one prior therapy, for the treatment of MM patients with previously untreated MM who are not eligible for high-dose chemotherapy with bone marrow transplant in combination with melphalan and prednisone, and for the treatment of patients with mantle-cell lymphoma who have received at least one prior therapy. An increasing number of studies have examined the effect of bortezomib in myeloma-related bone disease. However, there are no available data for the effect of proteasome inhibition by bortezomib on bone metastasis of solid tumors, despite the fact that the beneficial effect of bortezomib has been tested in both preclinical and clinical settings in a variety of solid tumors such as non-small cell lung, prostate and breast cancer.[30–32] The second-generation proteasome inhibitor carfilzomib, has recently been approved for treatment of relapsed and refractory MM patients who have received at least two prior therapies, including bortezomib and an immunomodulatory agent. As data for the effect of bortezomib on bone metastasis from solid tumors or the effect of carfilzomib on bone metabolism are very limited, this review will focus on the effect of bortezomib on myeloma bone disease.

In preclinical models, it was first reported by Giuliani *et al.*[33] that bortezomib could induce osteoblast differentiation in human mesenchymal cells, an effect associated with increased Runx2/cbfa1 expression but not Wnt signaling.

In another study, bortezomib induced osteoblast differentiation via Wnt-independent activation of β-catenin/T-cell factor (TCF) pathway suggesting that proteasome inhibition therapy in MM may function in part by subverting tumor-induced suppression of canonical Wnt signaling in the bone microenvironment.[34] Mukherjee *et al.*[35] demonstrated that bortezomib promoted an increase in the size of osteoblastic colony-forming units but had no effect on their number. Bortezomib stimulated osteoblast differentiation and bone formation in bone organ cultures in a BMP-dependent manner, in addition to inhibiting Dkk-1 expression in bone and bone-derived cells.[36] These data suggest that bortezomib may promote bone formation by stimulating progenitor proliferation and osteoblast differentiation. Treatment of nonmyeloma-bearing mice with bortezomib resulted in an increase in bone mineral density (BMD). In the severe combined immunodeficiency (SCID)-rab model of myeloma, in mice that were responsive to treatment, there was also an increase in BMD; however, this was not seen in nonresponsive mice, and the BMD decreased. The increase in BMD was not seen in dexamethasone-responsive mice and was associated with increased numbers of osteocalcin (OC)-expressing osteoblasts and reduced numbers of tartrate-resistant acid phosphatase (TRACP)-expressing osteoclasts.[37] In addition to promoting bone formation, bortezomib also affects osteoclast differentiation and function in a dose-dependent manner, thus reducing subsequent bone resorption. Bortezomib seems to act in both early and late phase of osteoclast differentiation, through the inhibition of the p38 mitogen-activated protein kinase pathways (early phase), activator protein-1 and NFκB signaling (late phase).[26] *In vivo*, bortezomib treatment of SCID-rab mice bearing myeloma was associated with a reduction in the osteoclast number.[38] However, separating the direct effects of bortezomib on osteoclasts and osteoblasts, and the indirect effects via the inhibition of myeloma cells *in vivo* is difficult. The interaction of myeloma cells and bone marrow microenvironment is crucial, as myeloma cells exhibit an enhanced response to bortezomib treatment, indicated by a greater reduction in tumor burden, when the myeloma cells are located within the bone marrow compared with extra-osseous sites.[38] Nevertheless, the concentrations of bortezomib used in these studies were typically less than that required to induce tumor cell apoptosis.

An increasing number of studies are reporting the effects of bortezomib on bone formation in the clinical setting, confirming preclinical observations. In two large bortezomib trials (SUMMIT and APEX), patients with relapsed MM who had a partial response to bortezomib therapy had a transient-increased bone-specific alkaline phosphatase (bALP) level compared with non-responders.[39] In another study by Heider *et al.*,[40] significant increase in serum concentrations of both bALP and osteocalcin was found in MM patients treated with bortezomib, but not in patients treated with other anti-myeloma agents. The increase in bALP was significant both in responders and non-responders to bortezomib, and this finding suggested

a direct effect of proteasome inhibition on osteoblastic activity. Terpos *et al.*[41] also showed that bortezomib significantly increased the serum levels of bALP and OC in 34 patients with relapsed MM. Patients who achieved a complete response (CR) or very good partial response (VGPR) after 4 cycles of bortezomib had greater elevations of bALP levels than those not achieving a CR or VGPR. Interestingly, 75% of the non-responders also had an increase in bALP levels following four cycles of bortezomib treatment. The increase in bone formation markers was accompanied by a reduction in Dkk-1 serum levels, which was similar among responders and non-responders after bortezomib therapy. In another cohort of patients, bortezomib monotherapy resulted in a reduction of sclerostin by almost 50% in both responders and non-responders,[14] indicating that the anabolic effect of bortezomib is mediated through the Wnt signaling pathway. The combination of bortezomib–dexamethasone plus zoledronic acid increased BMD in a subset of relapsed MM patients with low BMD and non-extensive lytic disease who received this regimen at first relapse.[42] This BMD improvement was observed very early, in 6 months post initiation of the therapy, and has not been described with other anti-myeloma regimens even in responding patients. The *post hoc* analysis of the phase III VISTA trial indicated that the addition of bortezomib to the combination of melphalan and prednisolone as frontline treatment for patients with MM ineligible for high-dose therapy seemed to be associated with a reduction in disease progression because of worsening bone disease, healing of bone lesion in some patients in the VMP arm, reduced Dkk-1 levels in the VMP arm, less bisphosphonates use and less need for subsequent radiotherapy, including the potential for radiologic bone healing.[43] However, in patients with relapsed/refractory myeloma, when bortezomib was combined with other anti-myeloma agents, such as melphalan, dexamethasone and intermittent thalidomide (VMDT regimen), no increase in bALP and osteocalcin was observed, suggesting that bortezomib in combination with other anti-myeloma agents may lose its beneficial effect on osteoblasts.[44] Even in post-autologous stem cell transplantation (ASCT) patients with low myeloma burden, bortezomib in combination with thalidomide and dexamethasone as consolidation therapy failed to produce a significant bone anabolic effect.[45] Both dexamethasone and thalidomide are known to reduce bone formation markers and thus bortezomib's bone anabolic effect seems not to override the negative impact of these drugs on osteoblast function.[46,47] Carfilzomib has also been reported to increase bALP in patients with relapsed myeloma who responded to therapy.[48]

Bortezomib also inhibits osteoclast activity. Results from a prospective study on markers of bone resorption by Uy *et al.*[49] suggest an inhibitory effect of bortezomib on osteoclastic bone resorption in patients receiving the agent as consolidation treatment following transplantation. Patients were treated with bortezomib once weekly for 4 of every 5 weeks, 90–120 days after transplantation, for 6 cycles and did not receive bisphosphonates from 42 days before stem cell collection until cycle 3 of bortezomib therapy. Following two cycles of consolidation therapy, a 32% reduction in urinary N-terminal crosslinking telopeptide of collagen type-I (NTX) excretion, a highly specific marker of bone resorption was observed, suggesting that bortezomib has an inhibitory effect on osteoclast activity and bone resorption, which was seen even in patients at a plateau phase of their disease. However, contrary to recent reports on the positive effect of bortezomib on osteoblastic bone formation, a decline in OC levels was observed in this study. In another study by Terpos *et al.* in post-ASCT patients with low myeloma burden who did not receive bisphosphonates during consolidation with bortezomib, thalidomide and dexamethasone, bone resorption as assessed by the serum levels of C-terminal crosslinking telopeptide of collagen type-I (CTX) was also reduced.[45] Furthermore, Terpos *et al.*[41] have reported in 34 patients with relapsed myeloma that bortezomib administration, at the standard dosage, resulted in a significant reduction in serum RANKL levels, after 4 and 8 cycles of therapy, with concomitant reduction in osteoclast function and bone resorption, as assessed by the serum levels of TRACP type-5b (TRACP-5b) and CTX, respectively. The reduction in osteoclast function and bone resorption occurred irrespective of response to therapy in this study.

These findings suggest that proteasome inhibition and especially bortezomib may also have beneficial effects on severe bone disease in addition to its antineoplastic effects on tumor cells. However, it is unclear if bortezomib alone is sufficient to reverse bone disease in MM patients and heal lytic lesions.

Conclusions

Bone disease is a major problem in the management of malignancies. Increased understanding of the pathophysiology of bone disease is helping to identify new therapeutic targets, including RANKL, sclerostin and activin-A, for this debilitating complication of solid tumors and MM. The ubiquitin–proteasome pathway is one system implicated in bone remodeling and in the growth and survival of cancer cells through the RANKL/OPG system and the Wnt signaling pathway. Bortezomib, the first-in-class proteasome inhibitor, has been incorporated in the treatment plans of both myeloma and lymphomas, while there is growing evidence for its efficacy in some types of solid tumors. The available data suggest that bortezomib regulates bone remodeling by inhibiting bone resorption and promoting bone formation, but it is difficult to separate the direct effects of bortezomib on osteoclasts and osteoblasts, and indirect effects via the inhibition of cancer cells *in vivo*. The effect on osteoblast implication in the metastasis niche is also under investigation. Still, in contrast to other antineoplastic agents, bortezomib is the first agent that combines potent anticancer activity with potential beneficial effects on bone disease. More proof for the importance of proteasome inhibition in bone cancer will probably soon become available, as novel and more potent proteasome inhibitors with better toxicity profile, such as carfilzomib (less neurotoxicity) find their way into clinical practice.

Conflict of Interest

Evangelos Terpos declares honoraria and advisory fees from Janssen-Cilag. Dimitrios Christoulas has no interests to declare for this paper.

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