Novel Bone-Targeted Strategies in Oncology

Sonia Vallet, Matthew R. Smith, and Noopur Raje

Abstract

Most patients with bone metastases experience skeletal complications, resulting in significant morbidity and increased risk of death. Although the use of bisphosphonates is a well-established form of supportive care treatment for bone metastasis, complications arising from long-term use require schedule optimization and a search for alternative strategies. Moreover, the scope of use of bone-targeted agents in oncology has widened to include therapy-induced bone loss and antitumor effects. Indeed, bone provides a permissive niche to tumor growth, and targeting the interactions within the bone microenvironment is a promising antitumor strategy. In addition, the pathogenesis of cancer-related bone disease has been partially unraveled with a focus on the anabolic bone compartment, and the rapid bench-to-bedside translation has resulted in the identification of novel therapeutically amenable targets. This review focuses on studies optimizing bisphosphonate use and recent clinical data on denosumab in the treatment of bone disease. We also provide data on trials that have evaluated the antitumor effects of bisphosphonates and summarize the most recent discoveries on the role of the bone niche in cancer development, with insights into the preclinical rationale and clinical assessment of novel antiresorptive and anabolic bone-targeted agents.

Scope of the Problem

Bone involvement is a common feature in solid and hematologic cancers. Multiple myeloma localizes to the bone in nearly all patients, and the incidence of bone metastasis in solid cancers ranges between 20% and 75% (1, 2). Disease-related skeletal complications result in significant morbidity due to pain, pathologic fractures and spinal cord compression (3). In addition, the occurrence of pathologic fractures increases the risk of death by 20% to 40% (4). Not only is bone involvement due to metastatic disease of significance, but the increasing incidence of therapy-related bone loss has widened the scope of use of bone-targeted agents in oncology (5). In addition, recent preclinical and clinical studies have suggested that bone provides a permissive niche to tumor cell growth, and targeting the interactions within the bone milieu may represent an important strategy to suppress tumor development (6, 7). Therefore, restoring a balanced bone environment may not only positively impact bone disease but may also help reduce tumor burden.

In this review, we provide an overview of novel, biologically based therapeutic strategies aimed at preventing and/or decreasing disease-related skeletal complications and discuss the evidence for antitumor effects from restored bone homeostasis.

Biology of Bone Disease and Metastasis

The skeleton is a dynamic organ that undergoes extensive remodeling throughout life. Modest changes in the kinetics of bone resorption and formation result in dramatic net effects on bone architecture (8). In the cancer setting, the cross-talk between tumor and bone cells disrupts normal bone homeostasis, leading to development of skeletal lesions (Fig. 1). Although activation of osteoclasts, the bone-resorbing cells, is a common feature in both osteolytic and osteoblastic bone metastasis, the development of either type of lesion depends on the effects on osteoblasts, the bone-forming cells. Inhibition of osteoblasts results in osteolysis, typical of myeloma and breast cancer metastasis, whereas osteoblast hyperactivity results in osteoblastic lesions seen in prostate and sometimes breast cancer metastasis (3, 9).

Several cytokines are up-regulated in the tumoral bone milieu promoting osteoclast activity. Receptor activator of NF-κB ligand (RANKL) and its antagonist, osteoprotegerin, regulate osteoclastogenesis, and disruption of this balance is demonstrated in many cancers (10, 11). Osteoclast differentiation and activity are also promoted by several tumor cell–derived osteoclast-activating factors, such as parathyroid hormone-related protein, interleukins, and chemokines (12–16).

Osteoblast inhibition induced by breast cancer and myeloma cells depends on contact-mediated RINX2 down-regulation (17) and secretion of inhibitory cytokines,
such as activin A and DKK1 (Fig. 1A: refs. 18–20). In contrast, down-regulation of DKK1 by prostate cancer-derived endothelin 1 induces osteoblast hyperactivity (21). Here, the uncoupled osteoblastic response to increased osteoclast resorption leads to disorganized new bone formation and osteosclerotic lesions (Fig. 1B).

The bone microenvironment creates a supportive niche for tumor growth. Osteoclasts and bone marrow stromal cells, along with extracellular matrix and cytokines stimulate tumor cell proliferation and confer chemoresistance (22, 23). Therefore, the reciprocal interactions between tumor cells, osteoclasts, osteoblasts, and bone marrow stromal cells represent an important but underexploited clinical target to restore physiologic bone remodeling and suppress tumor growth.

**Bisphosphonates: Alternate Dosing Schedules and Novel Indications**

Bisphosphonates are the only agents approved by the U.S. Food and Drug Administration for the treatment of bone metastases from solid tumors and multiple myeloma in conjunction with standard antineoplastic therapy (24). The inhibitory effects of bisphosphonates on bone resorption are long-lasting and persist after therapy discontinuation. Interestingly, a parallel decrease in bone formation has been observed with bisphosphonate treatment; this trend reverses with therapy discontinuation (25, 26). A recent in vivo study showed that high cumulative doses of zoledronic acid, in contrast to low cumulative doses, interferes with bone remodeling by inhibiting both osteoclast and osteoblast function and may influence the mechanical properties of bone (27). These profound effects on bone turnover may in part contribute to complications such as osteonecrosis of the jaw. In the oncologic setting, bisphosphonates have often been used on a monthly schedule for long periods, leading to high cumulative exposures. Myeloma patients with osteonecrosis of the jaw have a gene and protein profiling consistent with suppression of both bone resorption and formation (28). Moreover, a recent retrospective analysis of 4000 bisphosphonate-treated patients identified median dose and duration of treatment as significant risk factors for the development of osteonecrosis of the jaw (29). The American Society of Clinical Oncology guidelines for the use of bisphosphonates in myeloma-related bone disease have been recently updated to address concerns about osteonecrosis of the jaw (30), and new clinical trials are underway to optimize bisphosphonate administration schedule.
As shown in Table 1, bisphosphonates are currently being evaluated with alternate dosing and scheduling and with the use of bone biomarker–directed schedules. Monthly versus quarterly zoledronic acid schedules in breast cancer patients are evaluated in the OPTIMIZE trial (NCT00424983). Alternate dosing schedules of bisphosphonates based on markers of bone resorption are also under assessment. N-telopeptide of type 1 collagen (NTX) is a product of bone catabolism. Elevated NTX levels are highly predictive of skeletal complications (pain, fractures, and need for intervention; ref. 31) and are therefore useful to monitor response to antiresorptive agents. The BISMARK (NCT00458796) and Z-MARK (NCT00622505) trials aim at tailoring zoledronic acid treatment according to NTX levels in breast cancer and myeloma patients. These are important studies, and their results will validate the usefulness of NTX as a surrogate marker of bone resorption to predict skeletal-related events and tailor bisphosphonate therapy.

The most likely mechanism of action of nitrogen-containing bisphosphonates is inhibition of farnesyl pyrophosphate synthase in the mevalonate pathway (32). In addition to inhibition of signaling proteins, such as RAS-ERK (33), bisphosphonate antitumor effects are mediated by down-regulation of telomerase expression, induction of apoptosis, and inhibition of cell proliferation (34, 35). Bisphosphonates may play an antitumor role also by modifying the tumor microenvironment via antiosteoclast, anti-angiogenic, and immunomodulatory effects (36). Interestingly, zoledronic acid in combination with interleukin-2 achieved a clinical response in prostate cancer patients (37, 38). Preclinical studies suggest that the antitumor effects of bisphosphonates may rely on administration schedules. For example, metronomic zoledronic acid down-regulates vascular endothelial growth factor with stronger antiangiogenic effects (36), and sequential chemotherapy–zoledronic acid treatment induces synergistic cytotoxicity in both breast and prostate cancer cell lines (39). Clinical studies with pamidronate suggest a survival advantage to second-line–treated myeloma patients (40). More recently, improved overall survival has been shown in
Denosumab: A Novel RANKL-Targeted Therapy

The significant catabolic effects of RANKL on bone remodeling, in addition to its key pathogenic role played in many cancers, provide the rationale for the development of RANKL inhibitors, such as denosumab (AMG 162; Amgen, Thousand Oaks, CA), a subcutaneously injected neutralizing antibody against human RANKL (44). Denosumab induces a sustained inhibition of bone resorption markers (45). In oncologic patients with elevated NTX levels, despite bisphosphonate treatment, denosumab inhibited bone resorption and prevented skeletal-related events in a higher proportion of patients compared with bisphosphonate continuation (46).

Not only is denosumab a promising alternative for patients refractory to bisphosphonates, but recent studies suggest that a monthly dose of denosumab (120 mg/mo) is superior to zoledronic acid (4 mg/mo) in delaying time to first and subsequent skeletal-related events in breast cancer patients (Table 1, NCT00321464; ref. 47). A similar study in myeloma and solid cancers showed that the two agents were comparable (Table 1, NCT0033075; ref. 48). Notably, the incidence of adverse events, including infections and osteonecrosis of the jaw, did not differ between the two treatment groups. A large head-to-head study in castration-resistant prostate cancer showed the superiority of denosumab to zoledronic acid in the treatment of bone metastases (Table 1, NCT00321620).1

RANKL triggers bone metastasis by stimulating migration and homing of RANK-expressing epithelial and melanoma cancer cells (49). Increased RANKL expression is an independent predictor of recurrence and confers poor prognosis in renal and prostate cancer (50, 51). In preclinical models, inhibition of the RANKL pathway resulted not only in decreased bone metastasis and osteolytic lesions, but also a decrease in tumor burden and improved survival (52–54). Single-agent denosumab induced an 86% response rate in patients with giant cell tumor of bone, characterized by osteoclast proliferation among RANKL-expressing stromal cells (55). Denosumab has also been assessed for its antitumor effects in relapsed or refractory myeloma patients (n = 50; Table 2, NCT00259740; ref. 56). Although injections of denosumab 120 mg/mo induced stable disease in a subset of patients, the small number of patients enrolled in the study did not yield any conclusions on the antitumor effect of denosumab in myeloma. In the setting of hormone refractory prostate cancer, an ongoing randomized trial is assessing denosumab (120 mg/mo) versus placebo on bone metastasis-free and overall survival (Table 1, NCT00286091). Further studies in combination strategies or in a remission setting will be useful to clarify any antitumor effects.

Other Biologically Based Novel Drugs

Novel targets to inhibit osteoclast-mediated bone resorption

In addition to the RANKL/osteoprotegerin ratio imbalance, cancer-induced osteoclast activation is associated with secretion of several osteoclast-activating factors that represent important therapeutic targets (Fig. 2). Multiple myeloma cell–derived CCL3 stimulates osteoclast precursor fusion into active resorptive cells by interacting with the receptor CCR1 (57). Preclinical studies on inhibition of the CCR1/CCL3 pathway by antisense strategies and small molecule CCR1 inhibitors showed restoration of normal bone architecture together with interruption of osteoclast–multiple myeloma cell interactions (57, 58). Because CCL3 has a stimulatory effect on multiple myeloma cell growth, strategies to inhibit the CCL3/CCR1 pathway may also have an effect on tumor burden. Therefore, agents targeting the CCL3/CCR1 pathway may soon be assessed in the clinical setting.

Novel agents with both antitumor and antiosteoclast activity are the B cell–activating factor neutralizing antibodies. B cell–activating factor is an osteoclast-derived multiple myeloma growth factor, and its inhibition reduces tumor burden as well as lytic lesions in in vivo models of bone disease (59). Because the neutralizing BAFF antibody impairs multiple myeloma cell–bone marrow stromal cell interactions, the antiosteoclast activity observed in vivo may be mediated by reduction in multiple myeloma burden or decreased secretion of pro-osteoclast cytokines (60). Based on these promising data, ongoing clinical trials of B cell–activating factor neutralizing antibody (LY2127399) in combination with Velcade (Millenium Takeda Oncology, Cambridge, MA) are confirming the effects on bone lesions and tumor burden (Table 3, NCT00689507).

Bone resorption by mature osteoclasts relies on sequential steps that represent therapeutically amenable targets. Osteoclast adhesion to the bone surface is mediated by integrins, the most important being αVβ3, and subsequent formation of the ruffled border depends on the tyrosine kinase Src. Two orally active c-Src inhibitors are currently being studied in phase II and III clinical trials. AZD0530 is evaluated in breast and prostate cancer.

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1 Amgen, unpublished data.
patients in comparison with zoledronic acid (Table 3, NCT00558272). Single-agent dasatinib normalizes NTX and alkaline phosphatase levels in more than 50% of patients with metastatic castration-resistant prostate cancer (61). The combination with docetaxel suggests antitumor and antiosteoclast activity (62), and an ongoing phase III randomized trial is evaluating docetaxel with or without dasatinib (Table 3, NCT00744497). Bone matrix degradation is achieved via secretion of acid, depending on chloride channels and protonic ATP pumps, and release of collagenolytic enzymes, such as cathepsins (63). Cathepsin K is a lysosomal proteinase, whose knockdown induces an osteopetrotic phenotype (64). Inhibition of cathepsin K by Odanacatib reduced NTX levels similar to bisphosphonates in patients with metastatic breast cancer (65). These studies will provide novel antiresorptive agents for use in the setting of bone metastasis.

Targeting osteoblast function
To date, attention has largely been focused on targeting osteoclasts. However, osteolytic lesions in myeloma and breast cancer patients are associated with impaired osteoblast function leading to an imbalanced osteoclast/osteoblast axis. Therefore, therapeutic strategies aimed at restoring osteoblast differentiation are now being explored (Fig. 2).

DKK1 is a Wnt inhibitor that binds and sequesters the Wnt receptor subunit LRPs5, therefore inhibiting Wnt/β-catenin signaling. We have studied the anabolic effects of two clinical-grade DKK1 neutralizing antibodies, BHQ880 (Novartis International, Basel, Switzerland) and Mab B3 (Ely Lilly, Indianapolis, IN), in our preclinical model of human multiple myeloma bone disease. DKK1 inhibition increased bone formation, decreased osteolytic lesions, and provided antitumor activity (66, 67). A clinical trial combining BHQ880 and bisphosphonates in relapsed/refractory myeloma patients is currently ongoing (Table 3, NCT00741377). Other Wnt inhibitors may be involved in the pathogenesis of osteoblast inhibition by myeloma cells; in particular, sclerostin is an osteoblast inhibitor released by osteocytes. The promising bone anabolic effects of sclerostin antagonists in osteoporotic patients establish sclerostin as a novel appealing target in cancer-induced bone

### Table 2. Clinical trials evaluating antitumor effects of bisphosphonates or denosumab

<table>
<thead>
<tr>
<th>Trial</th>
<th>Patients</th>
<th>Agent and schedule</th>
<th>Endpoints</th>
</tr>
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<tbody>
<tr>
<td>NCT00295646, phase III (42)</td>
<td>Premenopausal, hormone receptor-positive breast cancer patients (stage I, II)</td>
<td>Anastrozole 1 mg/d or tamoxifen 20 mg/d, each with or without zoledronate 8 mg/mo for 3 y</td>
<td>Disease-free survival in each group Recurrence-free survival and overall survival in each group Assess whether zoledronate added to standard adjuvant therapy can decrease or even prevent bone loss Adverse events</td>
</tr>
<tr>
<td>NCT00072020, phase III (43)</td>
<td>Stage II or stage III breast cancer patients</td>
<td>Neoadjuvant or adjuvant chemotherapy and/or hormonal therapy with or without zoledronate iv monthly for six doses, every 3 mo for eight doses, and then every 6 mo for five doses</td>
<td>Disease-free survival as assessed annually for 10 y Time to bone metastases and distant metastases Overall survival SRE prior to development and following development of bone metastases Safety and toxicity of zoledronic acid</td>
</tr>
<tr>
<td>NCT00259740, phase II (56)</td>
<td>Relapsed or plateau phase multiple myeloma patients</td>
<td>Denosumab sc 120 mg days 1, 8, 15, and 29 and every 28 days thereafter</td>
<td>Proportion of subjects who have a complete response or partial response to treatment Overall safety profile of denosumab</td>
</tr>
<tr>
<td>NCT00286091, phase III (57)</td>
<td>Hormone refractory prostate cancer without bone metastasis at baseline</td>
<td>Denosumab sc 120 mg/mo</td>
<td>Time to first occurrence of bone metastasis or death from any cause Overall survival</td>
</tr>
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Abbreviations: iv, intravenous; sc, subcutaneous; SRE, skeletal-related event.
disease (68). Of note, however, the safety profile of these anabolic agents raises some concerns regarding potential tumorigenic effects. Indeed, tumor cells are characterized by active Wnt signaling, and inhibition of the β-catenin pathway reduces tumor burden (69, 70). Therefore, all anabolic agents acting on the Wnt/β-catenin pathway require further studies to exclude any stimulatory effects on tumor growth (7).

A novel mechanism of osteoblast inhibition involving the activin/SMAD2/DLX5 pathway has been recently identified. High bone marrow plasma activin A levels correlate with osteolysis and DLX5 down-regulation in bone marrow biopsies of myeloma patients (20). In vivo targeting of this pathway with an activin A inhibitor improves bone disease and reduces tumor burden in a humanized model of multiple myeloma (71). In case of osteoblastic metastatic lesions characterized by osteoblast hyperactivity and uncoupled bone formation, endothelin-1 inhibitors (ZD4054) are under study for their antistromal effects (72).

A different pathogenic mechanism is involved in the development of osteosclerotic lesions in prostate and breast cancer patients. Cancer cell–derived endothelin-1 stimulates osteoblast function via inhibition of DKK1 synthesis (21). Inhibition of endothelin-A receptor via atrasentan or ZD4054 impairs the tumor-induced osteoblast response in a preclinical model of prostate and breast cancer (73). However, in hormone-refractory prostate cancer, the effects of atrasentan on disease progression are discordant (74–76). In the setting of bone metastasis of prostate cancer, atrasentan and zoledronic acid did not prove synergistic in markers of bone metabolism (77).

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inhibition strategies (Table 3, NCT00554229). Another option to achieve osteoblast inhibition is provided by the radiopharmaceuticals $^{89}$Sr and $^{153}$Sm, which localize preferentially to areas of active bone formation (78). Not only do these two agents provide pain relief in patients with bone metastasis, they may also improve survival in combination with chemotherapy (79).

Although osteoclast activation is a common pathogenic event in bone disease, osteoblast activity determines the development of osteolytic or osteoblastic metastasis and therefore represents an important target. In order to treat bone disease and suppress tumor growth, future strategies will require a combination of antiresorptive and anabolic agents.

**Conclusions**

Bench-to-bedside translation in the field of bone-targeted agents will provide oncologists with several novel

**Table 3. Clinical trials validating novel bone-targeted agents**

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<tr>
<td>NCT00689507, phase I</td>
<td>Relapsed or refractory multiple myeloma</td>
<td>Dose escalation LY2127399 iv on day 1 and 1.3 mg/m$^2$ bortezomib iv on days 1, 4, 8, and 11 of each 21-day cycle</td>
<td>Pharmacokinetic/dynamic modeling of LY2127399 Safety and toxicity profile for LY2127399 in combination with bortezomib Response rate, duration of response, and time to progression of LY2127399 as a single agent and in combination with bortezomib</td>
</tr>
<tr>
<td>NCT00558272, phase II</td>
<td>Prostate or breast cancer with metastatic bone disease</td>
<td>AZD0530 daily oral dose compared with zoledronic acid</td>
<td>Bone resorption markers Safety and tolerability of AZD0530</td>
</tr>
<tr>
<td>NCT00744497, phase III</td>
<td>Castration-resistant prostate cancer</td>
<td>Docetaxel/prednisone ± placebo or dasatinib daily oral dose</td>
<td>Overall survival Rate of change in urinary N-telopeptide values Time to first skeletal related event Rate of change in pain intensity Time to prostate-specific antigen progression Safety and tolerability of combination</td>
</tr>
<tr>
<td>NCT00741377, phase I/II</td>
<td>Relapse/refractory multiple myeloma</td>
<td>Phase I: escalating doses of BHQ880 in combination with standard chemotherapy and zoledronic acid Phase II: BHQ880 in combination with standard chemotherapy; zoledronic acid will be added after the first 28 days of therapy</td>
<td>Phase I: Maximum tolerated dose and dose-limiting toxicity Phase II: Time to first SRE and change in bone markers for bone resorption and formation Safety profile of BHQ880 Immunogenicity of BHQ880</td>
</tr>
<tr>
<td>NCT00747123, phase II</td>
<td>Multiple myeloma with osteolytic lesions</td>
<td>Escalating doses of ACE-011 sc monthly versus placebo</td>
<td>Safety and tolerability of ACE-011 Change in biochemical markers of bone formation and resorption Incidence of SREs</td>
</tr>
<tr>
<td>NCT00554229, phase III</td>
<td>Castration-resistant prostate cancer and bone metastasis</td>
<td>ZD4054 orally 10 mg versus placebo</td>
<td>Overall survival, progression-free survival Tolerability and safety profile of ZD4054 Incidence of SREs and bone metastases</td>
</tr>
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</table>

Abbreviations: sc, subcutaneous; iv, intravenous; SRE, skeletal-related event.
therapeutic options for the treatment of bone metastasis. The identification of optimal parameters to assess therapeutic efficacy is therefore critical. Bone surrogate markers such as NTX levels have been used to evaluate the efficacy of novel bone-targeted agents. However, clinical endpoints such as number of bone metastases and survival rates remain important parameters to determine the therapeutic potential of novel agents in cancer-induced bone disease. Additionally, novel functional imaging modalities will play a role in the assessment of responses to these agents.

To understand the role of bone-targeted agents, it is critical to consider the role of antitumor strategies with effects on bone remodeling. For example, immunomodulatory drugs such as lenalidomide and pomalidomide exert anti-osteoclast effects (80, 81). Bortezomib, a proteasome inhibitor, stimulates osteoblast differentiation via up-regulation of RUNX2 and inhibits osteoclast development (82, 83). Therefore, the effects of bone-targeted drugs should be assessed in the context of bone effects of these novel antitumor agents.

The stage is set to study therapeutic strategies that not only inhibit cancer-related bone disease but also target the dissemination of cancer cells to sanctuary sites in the bone. Greater understanding of bone biology will inform these strategies, and novel biomarkers and functional imaging modalities will facilitate future drug development.

Disclosure of Potential Conflicts of Interest

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References


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