**Biology of Bone Metastases**

Rachel L. Theriault, MD, and Richard L. Theriault, DO, MBA, FACP

**Background:** Bone metastases cause morbidity and mortality in multiple malignancies. In addition to portending a dire prognosis, bone metastases cause bone pain, fractures, hypercalcemia, spinal cord compression, and other nerve compression syndromes. Improved understanding of the mechanisms that predispose tumor metastases to bone is needed to improve patients’ therapeutic options, maintain their quality of life, and improve their survival.

**Methods:** This review discusses selected preclinical and clinical data regarding bone metastasis development and cytokine/molecular interactions predisposing to bone metastases formation. Potential interventions for reducing bone metastases are also described.

**Results:** Biologic mechanisms resulting in metastases of tumor cells to bone are being studied. Among these are the RANKL pathway, osteoclast activation via cytokines (produced by tumor cell and cells in the bone microenvironment), interactions with transient and stromal cells in the bone microenvironment, and molecules such as PTHrP and endothelin-1. These molecules offer important opportunities for targeted interventions to decrease bone metastases-associated morbidity.

**Conclusions:** Knowledge of the pathophysiology of bone and cancer is developing rapidly. Relationships among cancer cells, bone-derived cells, and cytokines provide opportunities for the development of new interventions. Therapy targeting osteoclast/osteoblast interactions has proven benefit for patients with bone metastases.

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**Introduction**

Approximately 70% of patients who die of breast or prostate cancer also have had bone metastases. Patients with kidney, thyroid, and lung cancers also have higher incidence of bone metastases compared with other types of cancers, such as gastrointestinal malignancies, which uncommonly spread to bone. In addition to portending a dire prognosis, bone metastases cause significant morbidity including bone pain, fractures, hypercalcemia, spinal cord compression, and other nerve compression syndromes. Understanding the mechanisms that predispose tumor metastases to bone will improve the therapeutic options for patients, maintain quality of life, and perhaps improve survival. This review focuses on the mechanisms of bone metastases in breast cancer, prostate cancer, and multiple myeloma, as most of the existing research involves these diseases. However, it is important to note that other solid tumors, specifically lung, thyroid, and kidney, as mentioned above, have a relatively high incidence of bone metastases.
Approximately 30% to 65% of patients with metastatic lung cancers will develop bone metastases, as will approximately 47% of patients with advanced thyroid cancer and 30% of patients with advanced renal carcinoma. Bone metastases are a dire consequence and portend a poor survival, with a median of less than 6 months. The mechanisms involved in bone metastases of lung and other solid tumor malignancies include many of the same pathways, as discussed below.

Background

Normal Bone Physiology
To appreciate the bone-tumor microenvironment, a review of the basic structure and function of bone is useful. Bone is made primarily of type I collagen that is mineralized with hydroxyapatite crystals. Within these mineralized structures are numerous growth factors that are released upon bone resorption. Bone is composed of two structural types: (1) cortical bone, the dense outer layer of bone, responsible for supporting the weight of the body, and (2) trabecular bone, the more metabolically active porous matrix located within the ends of the bones. Trabecular bone has a higher turnover remodeling rate than cortical bone has. Bone is continually undergoing dynamic remodeling in a coupled and sequential process of osteoclast-mediated bone resorption and osteoblast-mediated bone formation. Many hormones and molecules are involved in the close cross talk among osteoblasts, osteoclasts, and other cells within the bone microenvironment. When stimulated by bone morphogenic proteins and other growth factors, mesenchymal stem cells in the bone marrow stroma proliferate and form pre-osteoblasts that differentiate into osteoblasts. Osteoblasts synthesize the collagenous precursors of bone matrix (osteoid) and regulate bone mineralization. Osteoblasts express several molecules that are important for bone regulation, including parathyroid hormone (PTH) receptors, prostaglandins, estrogen, vitamin D3, and various cytokines. In addition, they are directly involved with the control of osteoclast differentiation through expression of receptor activator of nuclear factor κB ligand (RANKL), and they also secrete osteoprotegerin (OPG), a decoy RANK receptor, which inhibits osteoclast formation. Recent research has shown the Wnt pathway to be a key regulator of osteoblast function and bone formation. Activation of the Wnt/B-catenin signaling pathway leads to increased bone mass.

Osteoblasts and stromal cells are sources of RANKL. The stromal cells secrete RANKL into the microenvironment, promoting the development and differentiation of osteoclasts. Osteoclasts express RANKL on their surfaces. When RANKL binds to the receptor RANK found on the surfaces of monocytes, in the presence of macrophage colony-stimulating factor (M-CSF), it promotes cellular fusion of several monocytes to form a multinucleated osteoclast.

Osteoclasts are derived from precursor cells in the monocyte-macrophage lineage, initially arising as inactive osteoclasts that are then activated by the interaction of multiple molecules within the bone microenvironment, including the balance of RANKL and OPG levels, with OPG indirectly inhibiting osteoclast formation by binding to RANKL. Osteoclast production is also stimulated by interleukin 6 (IL-6), IL-1, prostaglandins, and CSFs. Activated osteoclasts bind to bone matrix via integrin proteins and secrete acid and lysosomal enzymes that degrade bone.

Metastatic Tumor Bone Physiology
Multiple steps are involved in the metastasis of a primary tumor to any distant site. These include angiogenesis, which provides both nutritional support for tumor growth as well as a route for tumor cell migration, local invasion through the basement membrane (a hallmark characteristic of a metastatic cell), adhesion to vessel endothelium in the target organs, and extravasation into the tissue. These events are supported by tumor cell secretion of matrix metalloproteinases (MMPs) and cathepsin K. Tumor cells will establish metastases and grow at the distant site if the microenvironment is appropriate.

Two other groups of cells within the bone microenvironment contribute to the metastatic bone niche: stromal cells and transient cells. Stromal cells arise from mesenchymal cells in the marrow and include adipocytes, fibroblasts, and osteoblasts. They support the differentiation and proliferation of cancer cells via molecules such as vascular cell adhesion molecule (VCAM-1), syndecan-1, and matrix metalloproteinase 2 (MMP-2). Transient cells include erythrocytes, T cells, and platelets, all of which have been shown to aid tumor growth and metastases through various pathways and molecules.

Bone remodeling involves a plethora of growth factors, cell adhesion molecules, and cytokines that make it an attractive site for metastatic tumor cells. Osteoclasts also prime the bone microenvironment for tumor cell growth by causing bone resorption, releasing many of these potential growth stimulating molecules into the milieu. No definitive studies have linked increased bone resorption to increased tumor cell mass, but limiting bone resorption seems to reduce tumor burden in bone (though not in soft tissues.).

Bone metastatic tumors consist of four types of radiographically defined lesions: osteolytic, osteoblastic, osteoporotic, and mixed. Osteolytic lesions are characterized by the destruction of bone, generally recognized as a hole in the cortex on plain radiographic imaging. Osteoblastic lesions are characterized by excess deposition of new bone and appear radiographically as more dense bone, i.e., whiter than the surrounding bone, often referred to as “osteosclerotic” in appearance. Osteoporotic lesions appear as “faded” bone without discrete...
areas of cortical destruction or increased density in the radiographic picture; the cortical lesions may be best demonstrated on computed tomography (CT) with bone windows, while trabecular lesions may be best seen on magnetic resonance imaging (MRI) of bone. Radiographically mixed lesions comprise a combination of bone destruction and new bone deposition. Mixed lesions often have a central clear area of cortical lysis surrounded by an area of increased density (sclerosis).

Generally, one type of lesion will predominate. Osteolytic lesions are most common in breast cancers and multiple myeloma. They result from influences of the cancer cells as well as normal cells within the microenvironment. Bone metastases in prostate cancers tend to be osteoblastic in appearance with disorderly excess bone deposition. Endothelin-1 (ET-1) stimulates the formation of new bone through osteoblast proliferation, as does platelet-derived growth factor-BB (PDGF-BB), although the detailed mechanisms of this formation are still unknown.12-14

The highly vascular metaphyseal bone, composed predominantly of trabecular bone, appears to be the preferred site for bone metastases. The mechanics of its sluggish sinusoidal vascular supply give the hematopoietic cells as well as invading tumor cells ample opportunity to move in and out of the marrow. In addition, the endothelial cells lining the sinusoids express multiple adhesion molecules, including P-selectin, E-selectin, intercellular adhesion molecule (ICAM-1), and the previously mentioned VCAM-1, all of which play key roles by enhancing extravasation of tumor cells into the marrow.5

The bone microenvironment contains many elements that appear to favor the growth of metastases, including osteoclasts themselves, bone-stored insulin-like growth factor-1 (IGF-1), transforming growth factor beta (TGF-β), calcium, phosphate, the stromal cells, and RANKL expression within the stromal cells.15 The actions of these molecules and others are discussed below.

Physiologic Pathways of Metastases

As noted, multiple molecules and pathways have been implicated in the development and propagation of bone metastases, some specific to individual tumor types. Below are the pathways that have been elucidated in breast cancer, prostate cancer, and multiple myeloma, three of the most common tumors to result in bone disease. Once established in the bone, a vicious cycle is created among metastatic tumor cells, osteoblasts, and osteoclasts that facilitates increased bone turnover and the survival of metastatic cells (figure).

Breast Cancer

Guise et al16 developed a model of breast cancer metastases to bone, based on breast cancer cell overproduction of parathyroid hormone-related peptide (PTHrP). Tumor-derived PTHrP is a major inducer of osteoclastogenesis, and its expression is specific to the bone metastatic niche. PTHrP interacts with parathyroid hormone receptor 1 (PTH1R) to cause osteoblastic expression of RANKL, with a decrease in expression of OPG, leading to a net increase in osteolytic activity.1 Breast cancer cells often produce PTHrP, which binds to PTH receptors, thus directly activating stromal cell and osteoblast cell production of RANKL. RANKL then interacts with RANK, which is expressed on hematopoietic osteoclast precursors, inducing differentiation into mature osteoclasts. The excess activity of these mature osteoclasts results in bone degradation, with subsequent release of bone-stored IGF-1 and TGF-β. IGF-1 appears to play an important role in stimulating breast cancer cell growth and directed migration into bone by activating cascades of signaling molecules, including PI-3 kinase, Akt, and NF-kB. TGF-β promotes bone metastases by promoting the production of PTHrP, which results in a vicious cycle of increased osteoclastic bone resorption.15 Breast cancer cells also produce multiple cytokines, including IL-6, IL-11, prostaglandin E2, M-CSF, and tumor necrosis factor alpha (TNF-α), which act to promote bone metastases by inducing osteoclastogenesis and suppressing osteoblasts. IL-11 is expressed by stromal cells, and its expression is induced by TGF-β and PTHrP. Prostaglandin E2 leads to enhanced osteoclast formation via increased expression of RANKL.1

Multiple cell adhesion molecules, including integrins, cadherins, osteopontin (OPN), bone sialoprotein (BSP), laminin, and type IV collagen play a role in breast cancer metastases to bone. Breast cancer cells expressing integrins for matrix proteins such as type I collagen, OPN, and BSP likely have a preference for bone, since integrin expression is crucial for cancer cells to arrest in target organs. Breast cancer cells that express elevated levels of OPN and BSP show preferential metastases to bone.17 However, the detailed step-by-step mechanisms involved in bone metastases remain to be elucidated. Cadherins also appear to be involved in bone metastases, both promoting and suppressing. E-cadherin appears to act as a suppressor to cancer invasion and metastases, while N-cadherin has been shown to increase cell migration and metastases. In addition, cadherin-11 also appears to promote bone metastases. In addition to molecules released via bone resorption, the bone marrow stromal cells themselves support the homing, differentiation, and proliferation of cancer cells in the bone. Marrow stromal cells express cadherin-11 (OB-cadherin), which appears to enhance the homing of breast cancer cells to the bone as well as stimulate osteoclastogenesis. Stromal cells also express fibronectin, which plays a role in the dormancy of breast cancer cells in bone.15 Wnt signaling and dickkopfs (DKK1) also appear to be involved in breast cancer bone metastases.
**Prostate Cancer**

Unlike breast and lung cancers, prostate cancers form osteoblastic metastases. The processes by which this occurs are less well understood than those involving osteolytic metastases. Several factors are involved in the formation of these types of metastases, some unique and some previously noted in the discussion of breast cancer bone metastases. Tumor-derived peptide ET-1 is a major determinant of osteoblastic bone metastases in prostate cancer, though its precise mechanisms of action remain to be elucidated. It appears to stimulate osteoblast cell proliferation via the endothelin-A receptor (ETAR), leading to pathological bone formation. ET-1 has multiple downstream targets including IL-6, Wnt5a, connective tissue growth factor (CTGF), and RANKL. Also of note, ET-1 suppresses the negative regulator of the Wnt signaling pathway, DKK1. In addition, somewhat paradoxically, abundant expression of PTHrP is also found in prostate cancer bone metastases. This makes sense in light of the osteoblastic potential of the NH2-terminal fragments of PTHrP when activated via the ETAR pathway. Prostate-specific antigen is involved in inactivating the osteolytic effects of PTHrP; in fact, PSA-cleaved PTHrP is a potent stimulator of bone formation. Prostate cancers that metastasize to bone also express PTHrP, which is thought to play a role in the osteosclerotic bone metastases by stimulating bone resorption, a prerequisite for subsequent osteosclerosis. Other factors involved in the osteoblastic metastases of prostate cancer include TGF-β, fibroblast growth factor (FGF), bone morphogenetic proteins (BMPs), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-1), adrenomedullin, and proteases.

**Multiple Myeloma**

While multiple myeloma is a systemic disease, it is characterized by osteolytic lesions that are present in up to 80% of newly diagnosed patients. Bone remodeling is severely impaired in multiple myeloma. The bone loss due to excess osteoclastic activity is enhanced by severe perturbations in osteoblast cell function. Thus the inability of osteoblasts to make new bone and repair bone destroyed by osteoclasts results in severe bone “wasting” and an increased number of skeletal-related events. Again, there

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**Figure. —** The vicious cycle: osteoblasts are activated by PTHrP, leading to increased production of RANKL. RANKL interacts with RANK receptor expressed on hematopoietic osteoclast precursors, resulting in mature active osteoclasts. Mature osteoclasts resorb bone, causing the release by bone and cancer cells of bone-stored minerals and growth factors, including IGF-1, TGF-β, IL-6, and more PTHrP, thus stimulating tumor cell growth. Osteoprotegerin (OPG) can inhibit this process by binding to RANKL.
is some overlap with breast and prostate cancer in the involved molecules and pathways (although in multiple myeloma, the lesions are exclusively osteolytic). Factors involved in myeloma bone lesions include RANKL, OPG, IL-6, macrophage inflammatory protein-1 alpha (MIP1-α), and TNF-β. Increases in RANKL and MIP1-α (produced by myeloma cells) as well as decreases in OPG release (which is directly sequestered by syndecan-1 expressed by the multiple myeloma cells) lead to enhanced osteoclastogenesis. IL-6 is a potent stimulator of osteoclast formation (along with IL-1) and appears to promote multiple myeloma cell survival as well as regulate adhesion to marrow stromal cells. MIP1-α increases RANKL and IL-6-stimulated osteoclast formation, and it enhances B1 integrin-mediated adhesion between myeloma and stromal cells. While some controversy exists and further research is needed, it may be that multiple myeloma cells themselves may acquire the functional properties of osteoclasts and have the potential to directly degrade bone. In addition, multiple myeloma cells secrete DKK1, possibly interrupting interaction of Wnt with its receptors on osteoblast precursors, suppressing osteoblast differentiation. DKK1 also alters the ratio of Wnt3a-regulated OPG to RANKL in osteoblasts, favoring increased osteoclast function. A DKK1 neutralizing antibody, BHQ880, has been shown to increase bone formation and indirectly inhibit growth of multiple myeloma cells in the presence of bone marrow stromal cells.

The molecules and signaling pathways involved in breast cancer, prostate cancer, and multiple myeloma, as well as their roles and results, are listed in Table 1.

**Clinical Applications**

As the mechanisms involved in bone metastases are further elucidated and the pathways involving many of the previously noted molecules are more clearly understood, the clinical applications of this knowledge can have a significant impact. As previously mentioned, treatment for bone metastases, including surgery, radiation and bone-modifying agents such as bisphosphonates and denosumab, have been palliative in nature, attempting to slow disease progression, palliate symptoms, and increase survival. Some of the molecules are disease-specific, but many appear to be part of a final common pathway that directly increases the number and activity of osteoclasts leading to bone destruction. The development of targeted agents for specific molecules in this final common pathway, as evidenced by denosumab and the RANKL pathway, has the potential to not only palliate but also possibly prevent the development of bone metastases and prolong patient survival.

**Treatment and Prevention of Bone Metastases**

Traditional treatment options for bone metastases include surgery, radiation (both targeted external-beam radiation and systemic radionuclide therapy), chemotherapy, endocrine therapy, and bisphosphonates. Systemic administration of therapeutic radioisotopes such as strontium-89 and samarium-153 have been shown to safely and effectively palliate painful bone metastases associated with multiple types of malignancies. There is some suggestion that they may have a tumoricidal effect as well. However, while the majority of research shows that surgery and radiation can improve quality of life and overall survival, they do not target the underlying pathophysiology of bone disease.

**Bisphosphonates**

Despite their seeming differences, both osteoblastic and osteolytic metastases are treated with drugs to reduce or block osteoclast activity and inhibit bone resorption. These are not curative treatments; they slow progression of lesions rather than restore bone health. Bisphosphonates are analogs of pyrophosphates that bind to remodeling bone, inhibiting several components of the bone resorptive process that include promotion of osteoclast apoptosis, inhibition of osteoclast formation, and recruitment. Older bisphosphonates such as clodronate do not contain nitrogen and are metabolized by osteoclasts. Later-generation bisphosphonates, like zoledronic acid and pamidronate, are nitrogen-containing agents that are internalized by the osteoclasts and inhibit their function by inhibiting farnesyl-diphosphonate (FPP) synthase.

Thus, bisphosphonates act by binding to bone surfaces undergoing remodeling and are internalized by osteoclasts during bone resorption, resulting in inhibition of osteolysis. The inability of bone to regenerate following bisphosphonate therapy supports the in vitro observation that breast cancer cells alter osteoblast function in addition to inhibiting osteoclast activity. Bisphosphonates are widely used for bone metastases in breast cancer, multiple myeloma, lung cancer, and prostate cancer (which also has a level of osteolytic activity). They have been shown to reduce bone-related skeletal events.

The US Food and Drug Administration (FDA) has approved pamidronate and zoledronic acid specifically for the treatment of bone metastases. However, other bisphosphonates, including ibandronate and clodronate, have been used with similar effects (Tables 2 and 3). The majority of studies of these drugs are randomized clinical trials using skeletal-related events (SREs) as primary endpoints. In these studies, SREs included bone pain, fractures, need for surgery or radiation for bone symptoms, spinal cord compression, and hypercalcemia. Among other studies, pamidronate has been shown in two large randomized placebo-controlled trials to decrease the skeletal morbidity rate, delay the time to first SRE, and significantly reduce pain. Zoledronic acid has also been shown to delay the time to first SRE,
<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Cytokines</th>
<th>Role</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>Parathyroid hormone-related peptide (PTHrP)</td>
<td>Interacts with PTHR1 to cause expression of RANKL</td>
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<td></td>
<td>Receptor activator of nuclear factor κB ligand (RANKL)</td>
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<td></td>
<td>Osteoprotegerin (OPG)</td>
<td>Acts as a decoy RANK receptor</td>
<td>Blocks RANK/RANKL interaction, inhibits osteoclast development</td>
</tr>
<tr>
<td></td>
<td>Insulin-like growth factor 1 (IGF-1)</td>
<td>Stimulates chemotaxis of cancer cells and directs migration</td>
<td>Causes proliferation of cancer cells in bone</td>
</tr>
<tr>
<td></td>
<td>Transforming growth factor beta (TGF-β)</td>
<td>Enhances production of PTHrP</td>
<td>Stimulates osteoclast-mediated bone resorption</td>
</tr>
<tr>
<td></td>
<td>Interleukin 6 (IL-6)</td>
<td>Induces osteoclastogenesis and suppresses osteoblasts</td>
<td>Leads to bone resorption, decreased bone production</td>
</tr>
<tr>
<td></td>
<td>Interleukin 11 (IL-11)</td>
<td>Induces osteoclastogenesis and suppresses osteoblasts</td>
<td>Leads to bone resorption, decreased bone production</td>
</tr>
<tr>
<td></td>
<td>Prostaglandin E2</td>
<td>Increases expression of RANKL leading to enhanced osteoclast formation</td>
<td>Stimulates bone resorption</td>
</tr>
<tr>
<td></td>
<td>Macrophage colony-stimulating factor (M-CSF)</td>
<td>Induces osteoclastogenesis and suppresses osteoblasts</td>
<td>Leads to bone resorption</td>
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<tr>
<td></td>
<td>Tumor necrosis factor alpha (TNF-α)</td>
<td>Induces osteoclastogenesis and suppresses osteoblasts</td>
<td>Leads to bone resorption</td>
</tr>
<tr>
<td></td>
<td>Integrins</td>
<td>Allows cancer cells to arrest in target organs</td>
<td>Allows proliferation of cancer cells in bone</td>
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<tr>
<td></td>
<td>Cadherins</td>
<td>Unknown mechanism</td>
<td>Involved in migration and invasion</td>
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<tr>
<td></td>
<td>Osteopontin (OPN)/bone sialoprotein (BSP)</td>
<td>Unknown mechanism</td>
<td>Leads to bone resorption</td>
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<td>Prostate</td>
<td>Endothelin-1 (ET-1)</td>
<td>Stimulates osteoblast proliferation</td>
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<td>Platelet-derived growth factor (PDGF)</td>
<td>Stimulates osteoblast proliferation</td>
<td>Leads to bone formation</td>
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<td>Stimulates chemotaxis of cancer cells and directs migration</td>
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<td>Multiple Myeloma</td>
<td>Receptor activator of nuclear factor κB ligand (RANKL)</td>
<td>Binds to RANK receptor on precursor osteoclasts</td>
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<td>Tumor necrosis factor beta (TNF-β)</td>
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<td>Leads to bone resorption</td>
</tr>
<tr>
<td></td>
<td>Dickkopfs (DKK1)</td>
<td>Inhibits Wnt signaling</td>
<td>Leads to increased bone resorption</td>
</tr>
</tbody>
</table>
reduce the incidence of SREs, and significantly reduce pain.\textsuperscript{2,25,26} Although not approved in the United States, clodronate and ibandronate are used in other countries and also have been shown to delay the occurrence of new bone events and to decrease pain.\textsuperscript{27-30} The primary side effects associated with bisphosphonate treatment include anemia, gastrointestinal symptoms (eg, nausea, vomiting, diarrhea, or constipation), fatigue, fever, weakness, arthralgias, myalgias and, less commonly, hypocalcemia. Renal toxicity has led to an FDA black box warning to measure creatinine before bisphosphonate administration. Osteonecrosis of the jaw is a recently described complication of bisphosphonate use and is most commonly associated with the amino bisphosphonates. Dental evaluation for patients is recommended.\textsuperscript{31-33}

**Other Bone-Targeted Therapies**

Denosumab is the most recently approved therapy for osseous metastases. It is a fully human monoclonal antibody to RANKL and is a potent inhibitor of osteoclastogenesis and suppression of bone resorption. It has been approved for the treatment of post-menopausal osteoporosis as well as bone metastases from solid tumors and multiple myeloma. A phase II dose-finding study by Lipton et al\textsuperscript{34} studied women with breast cancer-related bone metastases, randomly assigning them to 6 different cohorts, 5 of which included denosumab. The primary endpoint was the change in the bone turnover marker, urine N-telopeptide, corrected for urine creatinine (uNTx/Cr). Safety and SREs were also evaluated. A significantly higher proportion of patients on denosumab demonstrated a reduction in the uNTx/Cr, and significantly fewer patients on denosumab experienced SREs (9% compared to 16% on bisphosphonates). A large, placebo-controlled randomized trial directly compared denosumab to zoledronic acid and found that it was superior in delaying or preventing SREs in patients with breast cancer metastatic to bone.\textsuperscript{35}

Multiple other therapeutic agents are currently being investigated, including antibodies against PTHrP and pharmacologic agents that block PTHrP, which in experimental models block osteolysis.\textsuperscript{36,37} TGF-\textbeta is also a potential target, and inhibitors of TGF-\textbeta receptor 1 kinase have been shown to reduce the development and progression of osteolytic bone metastases through

### Table 2. — Treatment of Bone Metastases

<table>
<thead>
<tr>
<th>Agent</th>
<th>FDA-Approved Use</th>
<th>Other Uses</th>
<th>Study/Endpoint</th>
<th>Side Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pamidronate</td>
<td>Treatment of osteolytic bone metastases of breast cancer and multiple myeloma</td>
<td>Treatment of hypercalcemia of malignancy, Paget’s disease</td>
<td>DBRCT/SRE\textsuperscript{a}</td>
<td>Renal toxicity, osteonecrosis of jaw, hypocalcemia</td>
</tr>
<tr>
<td>Zoledronic acid</td>
<td>Treatment of multiple myeloma and bone metastases from solid tumors</td>
<td>Treatment of hypercalcemia of malignancy</td>
<td>Randomized controlled trial/SRE\textsuperscript{b}</td>
<td>Renal toxicity, osteonecrosis of jaw, fever, nausea, constipation, anemia, dyspnea, hypocalcemia</td>
</tr>
<tr>
<td>Ibandronate</td>
<td>Treatment and prevention of post-menopausal osteoporosis</td>
<td>Treatment of bone metastases, hypercalcemia of malignancy</td>
<td>DBRCT/skeletal morbidity period rate\textsuperscript{c}</td>
<td>Dyspepsia, arthralgias, hypocalcemia</td>
</tr>
<tr>
<td>Clodronate</td>
<td>None in United States</td>
<td>Osteoporosis, treatment and prevention of bone metastases in breast cancer</td>
<td>RCT/new bone metastases</td>
<td>Dyspepsia</td>
</tr>
<tr>
<td>Denosumab</td>
<td>Prevention of SREs in bone metastases from solid tumors</td>
<td>Prevention of postmenopausal osteoporosis</td>
<td>DBRCT/SRE</td>
<td>Urinary tract infection, upper respiratory infection, constipation, cataract, arthralgias, hypocalcemia, osteonecrosis of the jaw</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Skeletal-related event (SRE) is defined as a pathological fracture, radiation therapy to bone, surgery to bone, or spinal cord compression.

\textsuperscript{b} The number of 12-week periods with new skeletal complications.

\textsuperscript{c} DBRCT = double-blind randomized controlled trial.

### Table 3. — Guidelines for Use of Agents Approved for Treatment of Bone Metastases

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose</th>
<th>Administration</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pamidronate</td>
<td>90 mg</td>
<td>Intravenous, over a minimum of 2 hrs</td>
<td>Every 4 wks</td>
</tr>
<tr>
<td>Zoledronic acid</td>
<td>4 mg</td>
<td>Intravenous, over a minimum of 15 mins</td>
<td>Every 3–4 wks</td>
</tr>
<tr>
<td>Denosumab</td>
<td>120 mg</td>
<td>Subcutaneously in upper arm, upper thigh or abdomen</td>
<td>Every 4 wks</td>
</tr>
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a variety of downstream effects.\textsuperscript{39} Cathepsin K is a molecule that is produced by metastatic cancer cells and is involved in osteoclast-mediated bone degradation. In preclinical models, inhibitors of cathepsin K have been shown to reduce tumor burden in breast cancer metastatic to bone. A recent double-blind, randomized controlled trial reported by Jensen et al\textsuperscript{39} showed that odanacatib, a cathepsin K inhibitor, suppressed uNTx similarly to zoledronic acid and was safely tolerated. PSK 1404, an antagonist of alpha vB3 integrin, an adhesion receptor implicated in tumor cell invasion and osteoclast-mediated bone resorption, has also been shown in preclinical models to reduce bone destruction and skeletal tumor burden.\textsuperscript{40} The ET-1 receptor is also a potential for targeted therapy; preclinical data involving ETAR antagonists that inhibit the ET-1 signaling pathway have been shown to reduce tumor burden and bone lesions.\textsuperscript{41} Atrasentan is an ETAR antagonist that has been shown to delay progression of osteoblastic skeletal metastases in men with advanced prostate cancer.\textsuperscript{18,42} ZD4054, or zibotentan, is another ETAR antagonist that is currently in clinical trials. Another possible target is the suppression of endogenous antagonists of the Wnt signaling pathway, suppressing antagonists such as DKK1 and sclerostin. The antibody BHQ880 is a fully human monoclonal antibody that promotes osteoblastogenesis.\textsuperscript{20} Several drugs currently used in the treatment of multiple myeloma, including the pro tease inhibitor bortezomib and immunomodulatory drugs lenalidomide and pomalidomide, are being investigated further as they have been shown to inhibit osteoclastogenesis in vitro and to decrease the RANKL/OPG ratio in patient serum.\textsuperscript{43,44} As the common and disease-specific pathways involved in bone metastases are further elucidated, more targets will become available to intervene clinically.

**Prevention of Bone Metastases**

In addition to the treatment of existing bone metastases, there is significant interest in the potential prevention of their development. For breast cancer, several researchers have investigated the effects of adjuvant bisphosphonate treatment with clodronate in patients with primary breast cancer. Diel et al\textsuperscript{45} showed that in patients with primary breast cancer and evidence of tumor cells in the bone marrow, adjuvant clodronate for 2 years decreased the number of both visceral and osseous distant metastases, and it also decreased the number of bone metastases. Later, Powles et al\textsuperscript{46} showed a significant decrease in bone metastases during the treatment period and a significant reduction in mortality during the follow-up period. In contrast, Saarto et al\textsuperscript{47,48} also studied adjuvant bisphosphonate treatment for patients with node-positive breast cancer, but their results showed no significant difference in the development of skeletal metastases. Surprisingly, lower overall and disease-free survival and more non-skeletal metastases were seen in the clodronate group. The study has been criticized for an imbalance in the randomization of ER/PR status, but the results stand true at a 10-year follow-up.

In addition, studies of zoledronic acid used in the primary breast cancer setting to prevent bone loss have shown a decrease in disease recurrence at all sites; thus, multiple studies are investigating its use in the prevention of metastases in multiple types of cancers.\textsuperscript{49} The NSABP B-34 trial is investigating whether clodronate, alone or in combination with other adjuvant therapies, in patients with early-stage breast cancer will improve disease-free survival. It was closed to accrual in March 2004 and results are pending. The Adjuvant Zoledronic Acid to Reduce Recurrence (AZURE) trial is a phase III, multicenter, randomized clinical trial evaluating the role of zoledronic acid in the adjuvant therapy of patients with stage II and III breast cancers. The recently presented results showed no overall differences in disease-free or overall survival. However, improved overall survival was seen in the postmenopausal group, suggesting the hypothesis that the adjuvant effects of bisphosphonates may be dependent on low estrogen in the bone microenvironment.\textsuperscript{50}

Studies also are ongoing to investigate the use of bisphosphonates in the prevention of bone metastases in other malignancies. The Zometa European Study (ZEUS), a randomized, open-label, multicenter study in Europe, is investigating the use of zoledronic acid in the prevention of bone metastases in high-risk prostate cancer patients; results are currently pending. The G2419 trial and the Non-Small Cell Lung Cancer Study US75 (Z-PACT) are ongoing, investigating the adjuvant use of bisphosphonates to prevent bone metastases in patients with non-small cell lung cancer.\textsuperscript{51}

**Conclusions**

While much is being discovered about the pathways involved in the pathophysiology of bone metastases in cancers, a plethora of questions remain unanswered. What exactly is the “metastatic signature” of primary tumor? Is there only one signature? What triggers the growth of some tumor cells within bone marrow while others remain dormant? Further elucidation of the pathways involved in bone metastases in different types of cancers and the development of therapeutic agents to target specific steps in these pathways are needed. In addition to improving the treatment of these debilitating metastases, the goal of preventing them is equally important. While there are logistical and mechanical difficulties in studying the marrow and bone, significant advances have been made over the past decades in our knowledge regarding bone metastases and their treatment. Research must continue as we strive to alleviate our patients’ suffering and reduce the morbidity and mortality caused by bone metastases.
References

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