

Future Treatment of Bone Metastases

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Abstract All bone surfaces are periodically remodeled by the coupled and balanced action of osteoclasts and osteoblasts, of which the activities are regulated by a variety of cytokines and growth factors. Patients with cancer metastatic to the skeleton often develop osteolytic bone lesions, in which the actions of osteoclasts and osteoblasts remain coupled, but become imbalanced in sites adjacent to the tumor. The result is net bone loss. Many cancers secrete osteoclast-stimulating cytokines, which increase bone resorption by osteoclasts. In turn, factors released from the bone matrix during osteolysis can stimulate tumor growth. In this so-called "vicious cycle," there are multiple sites that are targets for new bone-directed therapies. A variety of new agents for the treatment and prevention of osteolytic bone metastasis are currently being developed. These include new agents that inhibit osteoclast differentiation, bone adhesion, and osteoclast function. These new strategies have evolved from a better understanding of the interaction between tumor cells and cells in the bone marrow microenvironment. There is great promise that these new bone-targeted therapies can decrease the frequent skeletal-related events that greatly diminish quality of life of patients with bone metastases.

The skeleton is the most common site of tumor metastasis, and malignant bone pain is the most common cause of severe cancer-associated pain (1). Patients with bone metastases are at risk of skeletal-related events (e.g., fracture, need for radiation therapy, and surgery) that can result in decreased quality of life (2, 3). Among patients with bone metastases from solid tumors or multiple myeloma, the annual incidence of skeletal complications is in the range of 1.5 to 4.0 events per year.

Patients with cancer metastatic to the skeleton often develop osteolytic bone lesions, in which osteoclast and osteoblast functions typically become uncoupled as well as imbalanced. Although a degree of coupling persists, the tight relationship between bone cell functions is typically lost. These cancers secrete osteoclast-stimulating cytokines, which increase bone resorption by osteoclasts. In turn, factors released from the bone matrix during osteolysis can stimulate tumor growth. In this so-called "vicious cycle," there are multiple sites that are targets for new bone-directed therapies (Fig. 1).

Some tumors, such as prostate cancer, may secrete cytokines that stimulate adjacent osteoblasts to increase their bone-forming activity, resulting in osteoblastic lesions (Fig. 2). Disruption or imbalance of the bone remodeling activities

of osteoclasts and osteoblasts leads to the progressive deterioration of skeletal integrity that underlies skeletal complications.

Nitrogen-Containing Bisphosphonates

Nitrogen-containing bisphosphonates have been introduced into clinical practice during the last decade, and successive generations of more potent agents with higher therapeutic indices have been developed (2, 3). Bisphosphonates preferentially bind to bone surfaces that are undergoing active remodeling and are then ingested by osteoclasts during bone resorption, thereby blocking signal transduction in the osteoclasts. This causes reduced osteolytic activity and the induction of programmed cell death (i.e., apoptosis), resulting in inhibition of osteolysis. Zoledronic acid treatment results in a delay in skeletal events in patients with bone metastases from breast cancer, prostate cancer, other solid tumors, and multiple myeloma. There is room for improvement as skeletal events still occur and the cancer continues to progress in the skeleton.

Agents to Interrupt the Vicious Cycle

In the so-called vicious cycle, there are multiple sites that are targets for new bone-directed therapies. Parathyroid hormone-related protein is released from certain tumor cells (e.g., breast cancer) and, by direct and indirect pathways, induces bone resorption. In animal model systems, an antibody to parathyroid hormone-related protein (CAL) inhibits osteolytic destruction caused by cancer cells (4). A phase 1 study of CAL in patients with bone metastases has recently been completed.

The vicious cycle can also be interrupted by diminishing the activities of transforming growth factor- β and platelet-derived growth factor released from bone matrix that has

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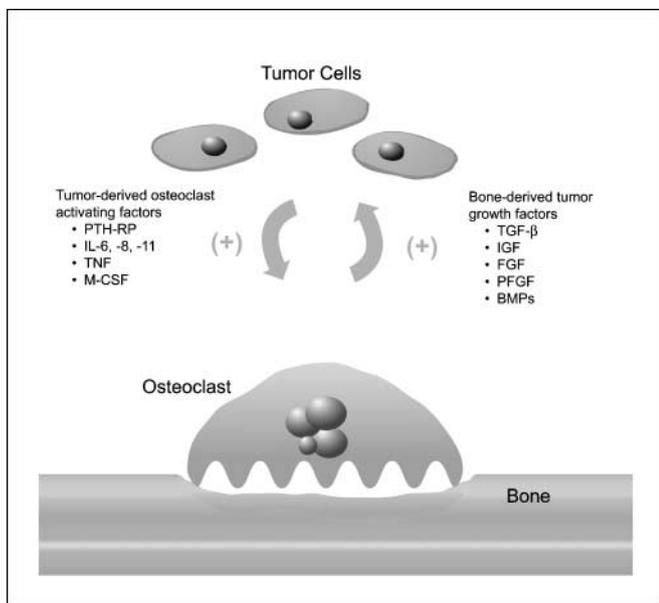


Fig. 1. Pathogenesis of osteoclastic metastases.

been resorbed by osteoclasts. Small-molecule inhibitors of transforming growth factor- β and imatinib mesylate, which inhibits platelet-derived growth factor receptor, can prevent osteolytic destruction in animal tumor models (5).

Inhibitors of Osteoclast Differentiation

Activated osteoclasts are found in proximity to tumor cells and play a key role in patients with multiple myeloma or cancer-related bone metastases. Normal homeostatic mechanisms are unable to control osteoclastic bone resorption. A triad of molecules has been shown to regulate the maturation, differentiation, and survival of osteoclasts: receptor activator of nuclear factor κ B (RANK), RANK ligand, and osteoprotegerin. RANK ligand, a member of the tumor necrosis family, binds to RANK on preosteoclasts and mature osteoclasts and mediates the differentiation, function, and survival of osteoclasts. Osteoprotegerin, a natural soluble decoy receptor of RANK ligand, modulates the effect of RANK ligand and is able to prevent excessive bone resorption (6, 7).

RANK ligand is a key mediator in the pathogenesis of a broad range of skeletal diseases. For example, tumor cells may express RANK, and tumor factors, such as parathyroid hormone-related protein, may up-regulate both RANK ligand and RANK expression in patients with cancer, causing excessive bone resorption. In particular, RANK ligand expression is elevated in patients with multiple myeloma and in some breast cancer cell lines. Denosumab (AMG 162) is a humanized monoclonal antibody to RANK ligand. Denosumab is in phase 3 trials in patients with metastatic disease in the skeleton and postmenopausal osteoporosis. As a human immunoglobulin G2 molecule, it has a long circulatory residence time and results in a rapid and sustained decrease of bone resorption in both healthy postmenopausal women and patients with bone metastases after a single s.c. injection (8, 9).

Inhibitors of Osteoclast Signaling and Enzymatic Activity

Cathepsin K. Cathepsin K is highly expressed in osteoclasts and has been identified as the crucial enzyme in collagen breakdown during bone resorption. Osteoclasts mediate bone resorption by removing both the calcium hydroxyapatite crystalline structure and protein matrix, which form the three-dimensional structure of bone. The protein matrix contains mainly collagen type I, which is destroyed by cathepsin K. The essential role cathepsin K plays in bone resorption is shown by data obtained from cathepsin K null mice, which display an osteopetrotic phenotype in the absence of any other overt pathologic signs. Point mutations in the gene that expresses cathepsin K result in pycnodysostosis, a rare bone disease characterized by increased bone density and osteosclerosis (10, 11). Inhibitors of cathepsin K effectively suppress bone resorption in animal tumor models (12, 13). Several small-molecule inhibitors of cathepsin K will soon enter phase 1 testing.

Src inhibition. Src is a tyrosine kinase that is required for the formation of the ruffled border in osteoclasts. The importance of Src in osteoclast activity is revealed by Src null mice that develop osteopetrosis because of the lack of bone resorption. Inhibition of Src activity decreases skeletal destruction in animal tumor model systems (14, 15). Phase 1 trials of Src inhibitors are being planned.

p38 mitogen-activated protein kinase inhibition. p38 mitogen-activated protein kinase is a member of the mitogen-activated protein kinase family of proteins that are involved in various growth factor and cytokine signal transduction pathways. p38 mitogen-activated protein kinase is involved in the production of interleukin 1 and tumor necrosis factor, two proinflammatory cytokines that promote osteoclast activity and bone resorption. Inhibitors of p38 mitogen-activated protein kinase decrease osteoclast activity in animal models (16, 17), and human trials are being planned.

Proteasome inhibition. Proteasome inhibitors have been shown to be potent stimulators of osteoblast function. In addition, proteasomes inhibit osteoclast differentiation by inhibiting nuclear factor- κ B signaling (18, 19). Although preclinical studies have shown bortezomib to decrease osteoclast function through inhibition of nuclear factor κ B, no clinical data exist on the effect of bortezomib on markers of bone resorption (20).

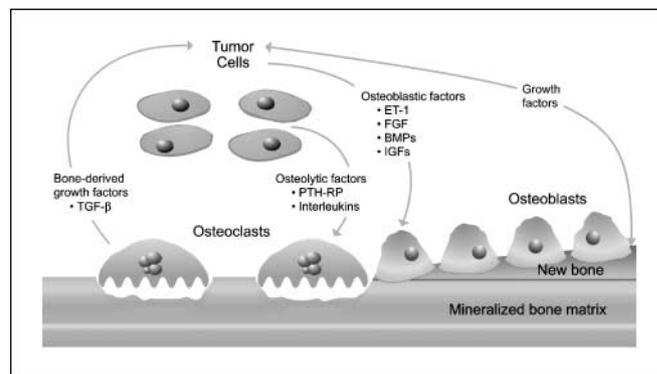


Fig. 2. Pathogenesis of osteoblastic metastases.

Inhibitors of Cell-Matrix Interaction and the Cytoskeleton

Mature osteoclasts must first adhere to the bone to mediate bone resorption. Vitronectin receptor, an $\alpha_v\beta_3$ integrin, is required for osteoclasts to adhere to the bone surface. Interactions between the osteoclast vitronectin receptor and the Arg-Gly-Asp tripeptide sequence found in several bone matrix proteins lead to osteoclast attachment, activation, and the release of cathepsins into the resorption lacuna. Several small-molecule inhibitors of the vitronectin receptor inhibit bone resorption *in vitro* and *in vivo* and may soon enter clinical testing (21, 22).

Angiogenesis Inhibitors

Angiogenesis is required for the growth of both primary and metastatic tumors and is dependent on an imbalance between stimulatory and inhibitory angiogenic factors. The expression of angiogenic factors is tissue specific, with each tumor having its own angiogenic phenotype. Human breast cancer metastatic lesions in mice express the angiogenic factors vascular endothelial growth factors A, B, and C and the osteoclast-inducing cytokines parathyroid hormone-related protein and macrophage colony-stimulating factor (23, 24). It is hypothesized that the angiogenic phenotype of breast cancer cells may be responsible for the pathogenesis of osteolytic bone metastases in breast cancer patients. Recently, a variety of angiogenic inhibitors (antibody and small-molecule inhibitors of vascular endothelial growth factor) have become available. To date, these have not been systematically tested in patients with bone metastases.

Endothelin Inhibitors

Endothelin-1 binds to the G-protein-coupled endothelin-A receptor and initiates signaling pathways that lead to vasoconstriction, cell proliferation, and angiogenesis. Endothelin-1 is highly secreted from prostate cancer cells and stimulates osteoblast proliferation, leading to osteoblastic bone metastases. Inhibiting the endothelin-A receptor may prevent the formation of osteoblastic metastasis in patients with prostate cancer. Atrasentan (ABT-627) is an inhibitor of the endothelin-A receptor and promotes bone formation *in vitro* and inhibits osteoblastic metastases in mice (25). Atrasentan is in phase 3 trials in patients with prostate cancer and bone metastases (26), as well as in patients with increasing prostate-specific antigen levels who are expected to develop bone metastases.

Conclusion

During the past decade, bisphosphonates have become established as the main treatment for patients with bone metastases from solid tumors and multiple myeloma. This treatment delays the rate at which skeletal-related events occur, but there is still much room for improvement. In the past few years, there has been much new understanding of the pathophysiology of bone metastases. This new knowledge opens the possibility of new treatments that could interfere with the tumor cell-osteoclast interaction. Studies in the near term will address the question of whether any of these new drugs will replace bisphosphonates as single-agent therapy of

bone metastases. A second goal will be to see if combination treatment of a new drug (e.g., a bone-seeking radiopharmaceutical such as samarium) plus a bisphosphonate is superior to bisphosphonate treatment alone. Furthermore, there is much hope that treatments can be developed that will prevent bone metastases.

Open Discussion

Dr. Berenson: We have to be careful about the design of trials. If we are going to look at drugs that may be either better or better with zoledronic acid, the companies that develop these drugs need to engage those of us who have studied this for many years rather than waste a lot of resources and time. We have to be careful in that development and be clear that the end point is something we're going to be able to reach.

Dr. Lipton: Do you have any advice?

Dr. Berenson: Perhaps some of these patients should be looked at as bisphosphonate resistant and then randomized once they've had an event, because they are likely to have a second event.

Dr. Roodman: There are data showing that patients who quit responding to one bisphosphonate will then respond to another bisphosphonate.

Dr. Clohisy: We spent about 2 years trying to study angiogenesis in renal cell and in trials of breast cancer in mice, and we found a few interesting things. One is that tumors that are highly angiogenic, in terms of proangiogenic gene expression in soft tissues, don't seem to be that way in bone, which has been disappointing. The other is that the antiangiogenic therapies alone have not been effective in bone unless they have been combined with radiation. In that setting, they have been very effective, particularly in the renal cell, almost completely eliminating the tumors.

Dr. Guise: When you say antiangiogenic therapy, is this specifically vascular endothelial growth factor-directed therapy? There are other factors that can contribute to angiogenesis and vascular endothelial growth factor therapy, but they may be problematic because they impair fracture healing.

Dr. Berenson: The other issue is that the effect of bevacizumab probably may have little to do with blood vessel development and a lot to do with changes and fluxes with drugs. There may be a lot of different things going on here than simply taking blood vessels away. In addition, there are a lot of other potential targets that are earlier. Vascular endothelial growth factor is a late target in angiogenesis.

Dr. Roodman: We have 80% inhibition of osteoclast activity in tumors that we can't make go away and continue to grow no matter what we've done. Some people say they can cure breast cancer metastatic to bone, but with prostate cancer, once it goes to the bone, it continues to grow most of the time. Is 80% inhibition enough? Do we need more?

Dr. Coleman: I'm not sure that's the correct way to look at the change in markers. It's 80% on average, but there are patients whose markers don't go down or hardly change.

Dr. Roodman: Right, 20%. But there are two ways to look at that data. The first is that we haven't inhibited their bone resorption; the second is that they have much more tumor or a more aggressive tumor, and it doesn't matter what you do because their tumor is going to continue to grow and induce osteoclastogenesis.

Dr. Lipton: Amgen is doing a study taking people with persistently high markers, who are taking zoledronic acid, and randomizing them and then continuing zoledronic acid versus AMG 162; so it will be interesting to see if it changes.

Dr. Roodman: That will be the critical question. I believe that enhancing the efficiency of antiresorptives, whether with a combination or a better drug, will affect it. However, we still have to worry about the fact that if we don't have something that kills the tumor, we may not do much better.

Dr. Boyce: How did you assess the vascular inhibition inside bone versus outside bone?

Dr. Clohisy: The outside bone is based on the same cell lines from other labs. For the inside bone, they measured microvascular density.

Dr. Body: You said that 20% of patients under prolonged bisphosphonate therapy keep an elevated N-telopeptide (NTX) level, but this has not been my experience. I am also involved in that Amgen trial and I guess only 5% of such patients had an elevated NTX level and could enter the trial. These markers are quite sensitive and they fall and normalize rapidly under bisphosphonate therapy, even if the process of tumor-induced osteolysis is going on, albeit at a slower rate. Therefore, we need other markers or some other means to evaluate this ongoing process of bone destruction. We would miss a lot of patients who are actually doing poorly if we just rely on the available bone markers and thus only treat by other agents the patients who do not normalize their NTX levels.

Dr. Smith: It's easy to reconcile the disparity between the high screen failure rate in the Amgen trial and the 20%, because

the 20% comes from a prospective evaluation of a supportive care trial, where patients could receive chemotherapy and be identified at some point as having elevated markers.

Dr. Lipton: The Amgen trial was very specific in that you had to be stable, that is, not requiring current chemotherapy. In other words, patients had to be doing extremely well but have high markers; therefore, the high screen failure rate is not at all surprising.

Dr. Coleman: In addition, those 15% to 20% with high markers were not always the same patients. These patients move in and out of the abnormal range, depending on what you've done to the underlying cancer.

Dr. Body: When I said 5%, I did not take into account the patients who were doing well with bisphosphonate therapy, because one expects that they would have a low NTX level anyway. My explanation is that there are biological fluctuations of the marker itself and also fluctuations of the marker according to the disease evolution. This is probably why you indeed get this 20% increased rate at certain time points in the disease course.

Dr. Smith: I didn't mean to suggest that it was investigator selection of those patients; it is that the eligibility criteria of the trial created a discordance.

Dr. Body: The important point, I think, is that you have many more patients with a normal NTX levels than the number of patients who are still progressing in bone and who would need another therapy. We would need specific markers of tumor-induced bone resorption to correctly identify these patients.

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