Bone homeostasis is primarily regulated by the interaction between the receptor activator of the nuclear factor kappa-β ligand (RANKL) secreted by the osteoblasts and its receptor (RANK) on the osteoclast precursors that leads to bone resorption. Osteoprotegerin (OPG) is a bone anti-resorption molecule that serves as a decoy receptor of RANKL, preventing the interaction between RANKL and RANK and thus inhibiting osteoclast activation. The RANKL–RANK–OPG axis is critical for the bone remodeling process that normally occurs in adult bone to preserve its function through aging. In the case of bone diseases such as osteoporosis or cancer, this dynamic balance between bone resorption and bone formation is interrupted, leading to either over-resorption or over-production of bone. In either case, the bone tissue is dysfunctional and prone to fractures, leading to severe pain and disability.

Denosumab (AMG 162) is a human monoclonal antibody that inhibits bone resorption by binding on the receptor activator of the nuclear factor kappa-β ligand, has recently emerged as an additional option in the treatment of musculoskeletal osteolytic tumors. This article focuses on the recent literature regarding the effectiveness of denosumab in the management of giant cell tumor, multiple myeloma, aneurysmal bone cyst, and osteosarcoma. The mechanism of action of denosumab in the management of these tumors and the associated side effects are discussed in detail. [Orthopedics. 2017; 40(4):204-210.]

Denosumab: Current Use in the Treatment of Primary Bone Tumors

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of the tumor necrosis factor family, prolactin, and corticosteroids.9

The receptor of RANKL, RANK, is expressed on the osteoclasts and their precursors. When RANKL binds to RANK, osteoclast activation is stimulated, resulting in bone resorption. Specifically, the extracellular receptor–binding domain of RANKL interacts with the extracellular cysteine-rich domain of RANK and activates it. The RANK activation results in the stimulation of several intracellular signaling pathways that finally lead to the activation of the osteoclastogenic genes in the nucleus. The transcription factor nuclear factor kappa-β is a key molecule in this sequence.10

On the other hand, OPG, which also belongs to the tumor necrosis factor family, binds RANKL, inhibiting its interaction with RANK on the osteoclastic or pre-osteoclastic cells. This results in inhibition of the osteoclast activation or differentiation and consequently prevents bone resorption.10

Increased activity of the osteoclastic bone signaling through the RANKL–RANK activation has been shown to be present in areas where metastatic bone lesions develop.11 This is especially true for cancers that result in osteolytic lesions when they metastasize to the bone (eg, thyroid and renal cancers). However, for cancers that finally yield metastatic osteoblastic lesions (eg, prostate cancer), animal studies have shown that excessive osteolysis occurs in the metastatic site before this overwhelming bone generation process begins.10,12 Therefore, because it is implicated in the balance between bone generation and resorption, the interaction of OPG with RANKL interferes with the type of bone metastasis arising as a complication of a primary tumor.11

The human monoclonal antibody denosumab was developed to bind RANKL, preventing its interaction with RANK. As a result, osteoclast activation, and consequently bone resorption, is inhibited. Dougall and Chaisson13 reported the potential of denosumab to treat metastatic bone disease. In phase 1 of a clinical trial, a single dose of denosumab successfully suppressed osteolysis in patients with multiple myeloma or bone metastasis from breast cancer. To identify possible therapeutic targets for denosumab, Yamagishi et al14 compared RANKL messenger RNA expression among 135 primary and metastatic bone tumors. Giant cell tumor of bone showed the highest expression of RANKL, followed by aneurysmal bone cyst, fibrous dysplasia, osteosarcoma, chondrosarcoma, and enchondroma, which also had relatively high levels. Schwarz and Ritchlin15 summarized the clinical trials conducted to seek approval of denosumab for the treatment of primary and metastatic bone tumors. The use of denosumab for the treatment of the latter is broad and beyond the scope of this article.

**Giant Cell Tumor of Bone**

Giant cell tumor of bone most commonly occurs during the third decade of life and has a slightly increased prevalence among females.16 It accounts for 5% of all primary and 20% of all benign bone tumors17,18 and has a predilection for the long bones around the knee, primarily the distal femur followed by the proximal tibia.18 Spine and pelvic bones may be affected. Giant cell tumor of bone has been reported to complicate Paget’s disease of the bone,19 and in such cases it may arise in the skull or spine. Histologically, giant cell tumor of bone is composed of multinucleated giant cells resembling osteoclasts, which are also referred to as osteoclast-like giant cells, and mononuclear stromal cells.20 It is generally considered a benign tumor; however, it is sometimes locally aggressive and can also metastasize to the lungs via the blood.21,22 However, these metastatic lesions are histologically benign and usually arise after therapeutic manipulations (mostly surgical) at the primary tumor area.22

The RANKL molecular pathway is highly implicated in the pathogenesis of giant cell tumor of bone. The RANK, which is highly expressed in osteoclast-like giant cells,23 is mainly responsible for the osteolytic behavior of this tumor. Denosumab, a RANKL inhibitor, was studied as a potential treatment for this tumor, being approved by the Food and Drug Administration for patients with unresectable giant cell tumor of bone or in cases in which surgery is contraindicated.24

Surgery involving complete excision–curettage is the gold standard therapeutic approach for giant cell tumor of bone, but the recurrence rate is relatively high. The recurrence rate initially ranged from 15% to 45%. However, it decreased to 12% to 14% with the use of a high-speed burr during curettage and the placement of allograft or cement for the post-resection bony defect.25,26 In certain cases, such as giant cell tumor of bone of the distal ulna or proximal fibula, en bloc bone resection of the tumor is feasible with a low local recurrence rate. Two medications currently used for bone tumors with osteoclastic activity, including giant cell tumor of bone, are bisphosphonates (mostly zoledronic acid) and denosumab.27,28 Denosumab is indicated as adjunct treatment to curettage and/or surgical resection of giant cell tumor of bone (Figure). It is also indicated in cases of large, unresectable, recurrent and/or metastatic giant cell tumor of bone to reduce morbidity associated with surgical intervention.29

Rekhi et al30 recently reported the clinicopathological effects of denosumab for 27 patients (27 tumors) with giant cell tumor of bone who underwent denosumab treatment followed by curettage and/or surgical resection. On histopathology, the osteoclast-like giant cells were completely eradicated in 55.5% (15 of 27) of the tumors, whereas residual osteoclast-like giant cells were present in 44% (12 of 27) after denosumab treatment. In addition, reactive woven bone was present in all posttreatment specimens. Similarly, Branstetter et al31 investigated the effect of denosumab for 20 adults with recurrent or unresectable giant cell tumor of bone. These patients received subcutaneous doses of 120 mg every 4 weeks, with additional denosumab administered on days 8 and 15. They had baseline and on-study biopsy or histologic specimens from resection. On histologic analysis following deno-
Because MMP-9 is involved in the degradation of the extracellular bone matrix, its inhibition prevents osteolysis. However, the changes in the levels of MMP-9 expression did not affect patient outcomes. In addition, this study showed that after denosumab therapy, the residual stromal cells can also cause osteolysis. Therefore, denosumab may need to be continued to prevent this complication.

However, these findings are contradictory to those of Lau et al, who compared the effect of denosumab vs zoledronic acid on giant cell tumor of bone stromal cells. They showed that denosumab caused minor inhibition of the growth of stromal cells and no apoptosis (0 of 3 cell lines). In contrast, the inhibition of growth by zoledronic acid was dose-dependent, and apoptosis occurred in 2 of the 3 cell lines. Further research is necessary to confirm the effect of denosumab on giant cell tumor of bone stromal cells.

Girolami et al studied the immunophenotypical changes in 15 cases of giant cell tumor of bone treated with denosumab. No significant changes in the expression of the RANK–RANKL molecules by the tumor cells were observed. However, denosumab significantly reduced the proliferation index (i.e., the number of cells in a tumor that are dividing) and the angiogenesis in these specimens.

The literature is limited regarding the clinical outcomes of patients with giant cell tumor of bone treated with denosumab. In a study of 27 cases of giant cell tumor of bone (18 primary and 9 recurrent) with an average follow-up of 17.6 months (80% follow-up rate for 25 of 27 patients), 20 (80%) of 25 patients were disease-free, while 5 (20%) of 25 patients had recurrence at 9 to 18 months. Curettage after denosumab therapy was performed in 15 cases, while the remaining 12 cases underwent post-denosumab surgical resection. Interestingly, the patients who had recurrence of the malignancy did not initially have unresectable or recurrent tumor. In a recent retrospective study, denosumab was administered to 18 patients to treat primary (n=11; 61%) or recurrent (n=7; 39%) giant cell tumor of bone. Ten patients who had unresectable tumors did not show disease progression after 18 to 60 months of denosumab administration. This study also identified positron emission tomography with 2-deoxy-2-[fluorine-18]fluoro-D-glucose integrated with computed tomography as a useful tool for assessing patient response to denosumab treatment. A phase II trial including 17 patients who received denosumab for giant cell tumor of bone showed 88% objective best (according to the criteria classification) response to therapy using any tumor response criteria and 35% using individual response criteria. Thus, denosumab was shown to elicit a relatively good therapeutic response in most of the patients.

Deveci et al prospectively evaluated radiologic response, symptoms (pain), and level of function at a mean follow-up of 17 months for 13 patients who received denosumab for giant cell tumor of bone and had satisfactory clinical outcomes. After 9 months of treatment, all patients had a 90% or greater decrease in the osteoclast-like giant cell population, whereas 65% (13 of 20) of the patients had development of dense fibrous tissue and/or new bone.

Müller et al operated on 25 patients who received denosumab treatment preoperatively and/or postoperatively. They reported one case of tumor recurrence. In all cases, denosumab reduced tumor size and provided a new peripheral margin, thus facilitating surgical treatment. In 16 cases, tumor resection, instead of curettage, was initially considered due to large tumor size. However, in 10 of these cases, treatment with denosumab provided a peripheral margin and changed the indication to curettage, which resulted in surgical downstaging.

Physicians must pay attention when performing a histopathologic examination of giant cell tumor of bone previously treated with denosumab. Because it may resemble osteosarcoma, more than a patient history is necessary to avoid misdiagnosis. Therefore, highlighting the histopathological differences between giant cell tumor of bone exposed to denosumab and osteosarcoma is important. Unlike osteosarcoma, giant cell tumor of bone treated with denosumab is MDM2 proto-oncogene negative. Moreover, giant cell tumor of bone exposed to denosumab presents a unique pattern of intralesional bone deposition that differentiates it from both osteosarcoma and giant cell tumor of bone not exposed to denosumab.

A proteomic analysis performed on giant cell tumor of bone samples before and after denosumab therapy showed that the matrix metalloproteinase (MMP)-9 was one of the most downregulated proteins after denosumab treatment. Because MMP-9 is involved in the degradation of the extracellular bone matrix, its inhibition prevents osteolysis. However, the changes in the levels of MMP-9 expression did not affect patient outcomes. In addition, this study showed that after denosumab therapy, the residual stromal cells can also cause osteolysis. Therefore, denosumab may need to be continued to prevent this complication.

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Systemic manifestations of patients with giant cell tumor of bone treated with denosumab, recent studies have shown denosumab to be beneficial.

**Multiple Myeloma Bone Disease**

Multiple myeloma, a disease of older individuals, is the second most common hematologic malignancy. Systemic manifestations, such as hypercalcemia and renal failure, may appear earlier in patients with multiple myeloma because there is a particularly intense osteoclastic activity compared with other bone tumors. This is due to the fact that in addition to the RANKL–RANK–OPG pathway, osteolysis in multiple myeloma is mediated via the tumor necrosis factor-related activation-induced cytokine–OPG axis. This molecular pathway is partially regulated by the tumor necrosis factor-alpha molecule. An increased soluble RANKL/OPG ratio in patients with multiple myeloma is an index of severe bone disease. It has a positive correlation with osteoclastic markers and predicts prolonged tumor survival and a worse clinical prognosis. Because denosumab can inhibit RANKL activity and oppose its osteoclastogenic effect, the potential of denosumab to treat or prevent multiple myeloma bone disease has been recently advocated. The effect of denosumab on the tumor necrosis factor-related activation-induced cytokine–OPG axis and its potential to counteract the action of this axis have not been investigated. Theoretically, this accessory osteolytic pathway could potentially decrease the overall anti-osteolytic effect of denosumab for patients with multiple myeloma. Studies are needed to confirm this hypothesis.

Bisphosphonates are widely used to prevent and treat myeloma bone disease. They inhibit the progression of bone lytic lesions; however, they do not induce bone regeneration in preexisting bone defects. Therefore, the effect of denosumab vs bisphosphonate on bone regeneration has been investigated. More specifically, alteration of the levels of several bone resorption and bone formation markers was studied in 10 patients with multiple myeloma who were administered denosumab after a period of initial therapy with zoledronic acid. A significant decrease in bone resorption markers (tartrate-resistant acid phosphatase 5b and serum type-I collagen cross-linked N-telopeptide) was observed; however, the levels of bone formation markers (osteocalcin and bone-specific alkaline phosphatase) were not significantly changed after treatment with denosumab. The response of multiple myeloma bone areas, including the bone volume, bone density, and multiple myeloma-cell density, to denosumab vs pamidronate was investigated in a computer model study. Denosumab led to improved bone formation compared with pamidronate. Thus, denosumab could be a useful alternative therapy to bisphosphonates in patients with multiple myeloma. Denosumab was compared with zoledronic acid regarding the prevention of skeletal-related events in a phase III clinical trial of 1776 patients with multiple myeloma, bone metastasis, or solid tumors. Of these patients, 180 (10%) had multiple myeloma and were assigned to one of two groups: 93 patients received 4 mg of zoledronic acid every 4 weeks, and 87 patients received 120 mg of denosumab subcutaneously. Mean follow-up time was 18.4 months and 17 months for the zoledronic acid group and the denosumab group, respectively. There was no difference regarding the skeletal-related events at the primary end point of time between the two groups. In contrast, an overall survival analysis involving the 180 patients with multiple myeloma showed zoledronic acid to be superior to denosumab (hazard ratio, 2.26). To better understand the difference in survival rates between the two groups, further analysis was conducted. More patients in the zoledronic acid group than in the denosumab group had an Eastern Cooperative Oncology Group score of 0 (32% vs 24%). In addition, the zoledronic acid group had a higher rate of International Staging System stage I multiple myeloma than the denosumab group (14% vs 10%). Although these parameters were not statistically significant, they partially explained the difference in survival rates. Although it was hypothesized that denosumab would be superior to bisphosphonates regarding bone regeneration and decreased skeletal-related events for patients with multiple myeloma, the difference between the two medications did not appear to be clinically significant. Research is needed to better characterize the role of denosumab in treatment for multiple myeloma and to compare its effectiveness with that of bisphosphonates.

**Aneurysmal Bone Cyst**

An aneurysmal bone cyst is a benign neoplasm that typically arises in the metaphysis of long bones such as the femur, tibia, and humerus. The vertebrae can also be affected; in such cases, neurological manifestations may be apparent. Although most aneurysmal bone cysts are primary lesions, 30% occur secondary to other bone tumors, including osteoblastoma, low-grade osteosarcoma, giant cell tumor, or fibrous dysplasia. Lately, the activation of the ubiquitin carboxyl-terminal hydrolase 6 oncogene in chromosome 17 is considered the pathophysiologic mechanism responsible for aneurysmal bone cyst development. Aneurysmal bone cysts were initially considered non-neoplastic; however, their as-
sociation with the ubiquitin carboxyl-terminal hydrolase 6 gene activation categorizes them as neoplastic disease. Aneurysmal bone cysts can be further characterized as inactive, active, or aggressive tumors based on their ability to expand, recur, and/or destroy surrounding healthy tissue. 60

Because aneurysmal bone cysts express osteoclastic markers, anti-osteoclastic agents such as denosumab have been suggested as potential therapy. Initially, Lange et al 52 administered denosumab to two patients with recurrence of spinal aneurysmal bone cysts after surgical resection. On magnetic resonance imaging, tumor regression was noticed in both patients after 2 and 4 months of treatment with denosumab. Pauli et al 47 treated an aggressive, recurrent aneurysmal bone cyst of the radius using denosumab as an adjunct. This made the tumor better circumscribed and thus amenable to complete resection. Histology showed a significant decrease of the osteoclastic giant cells and production of bone matrix. 47 Denosumab can be an alternative, non-operative treatment for aneurysmal bone cysts located at the spine 48,53 and for cases in which selective arterial embolization is not feasible. 54 Further research is needed to evaluate the outcome when denosumab is used to manage aneurysmal bone cysts.

Osteosarcoma
Osteosarcoma, the most common primary malignant bone tumor of younger individuals, arises primarily from osteoblasts. Inactivation of retinoblastoma and/or p53 tumor suppressor genes constitutes the pathophysiologic basis of osteosarcoma. The 5-year survival rate differs significantly between patients with localized (60% to 80%) vs metastatic (15% to 30%) osteosarcoma. Early detection and treatment of this tumor is critical for the prognosis. Osteosarcoma has the ability to cause erosion of the surrounding tissues or to metastasize to the lungs as a result of its osteoclastic properties. 35 The SaOS-2 human osteosarcoma cells have been shown to express RANKL and therefore support osteoclastogenic pathways. 36 Preclinical studies involving osteosarcoma animal models have shown that anti-RANKL agents decrease the growth of the tumor and counteract its metastatic potential. Based on this, denosumab has been implied to be a medication to prevent and/or treat osteosarcoma. 33,55 Regarding the use of denosumab in the management of osteosarcoma, there has been one case report. 57 A 37-year-old man with unresectable osteoblastic-like osteosarcoma in the C7/Th1 vertebra was treated with sorafenib and denosumab, which led to complete remission for more than 18 months.

Complications of Denosumab Therapy
Denosumab has been associated with side effects that primarily affect the osseous tissue, the immune system, and calcium homeostasis. 15,58 These complications can occur during or following discontinuation of treatment. Tumor transformation to a different malignant phenotype is another concern when using denosumab in orthopedic oncology.

Osteonecrosis of the jaw caused by non-bisphosphonate therapy has been characterized as anti-resorptive–related osteonecrosis of the jaw, and the risk for this complication is considered higher in patients who undergo dental procedures while receiving denosumab or other anti-resorptive treatment. 59 Owosho et al 60 reported 13 cases of osteonecrosis of the jaw among patients who were receiving denosumab monotherapy for metastatic bone tumors. Dental extraction prior to osteonecrosis of the jaw was reported in 7 patients. Discontinuation of denosumab resulted in complete resolution of osteonecrosis of the jaw in only 3 patients (23%), while 4 patients (30.7%) had progression. Osteosclerosis in most of the metaphyseal bones occurred in a 10-year-old boy who received five cycles of denosumab therapy for a giant cell tumor of the sacrum. 61 This complication was more prominent in the distal radius, ulna, proximal humerus, proximal femur, and phalanges of the fingers. Atypical femoral fractures were reported in 2 patients who were administered denosumab for the treatment of metastatic bone disease. 62

Schwarz and Ritchlin 15 reported that RANKL–RANK serves as a co-stimulatory molecule during T-cell activation; therefore, inhibition of RANKL using denosumab can result in immunosuppression. However, the RANKL–RANK pathway plays a role in T-cell activation only when the CD40–CD40 ligand signaling, which augments the interaction between T and B lymphocytes, is not present. Regarding the function of humoral immunity, the inhibition of the RANKL molecule does not seem to play a role. 63 A case of immunologic interstitial nephritis, confirmed with renal biopsy, was reported in a 76-year-old woman who was hospitalized for renal dysfunction 1 month after a single injection of denosumab for a osteoporosis-induced femoral fracture. Additional medications included bisoprolol and lysine asparin. Denosumab was discontinued; the patient was administered corticosteroids for 5 weeks, with complete restoration of renal function in 3 months. 64

Denosumab affects the bone generation–resorption balance and subsequently alters serum calcium levels. During denosumab therapy for 52 patients with metastatic bone disease, 9 (17.3%) had an episode of hypocalcemia, which was defined as calcium levels below 2 mmol/L. 58 The median number of denosumab injections before the episode was 1 (range, 1-14). Body et al 65 reported that the incidence of grade 2 or higher hypocalcemia in patients with bone metastasis was more than 2 times higher for those who received subcutaneous denosumab vs intravenous zoledronic acid (12.4% vs 5.3%). Patients who were taking calcium and/or vitamin D supplements had decreased incidence.

Rebound hypercalcemia can occur in patients after discontinuation of denosumab therapy. A calcium level of 16.5 mg/dL was detected in a 14-year-old girl 5 months after completion of denosumab therapy for a giant cell tumor; during treatment, the
calcium level was no higher than 8.4 mg/dL. A similar case was reported involving a 10-year-old boy with unresectable giant cell tumor of the sacrum who received denosumab. Four months after the discontinuation of therapy, the calcium level was 15.2 mg/dL and a significant rise in osteoclastic markers was detected. Bisphosphonate therapy was started to prevent further osteolytic damage. Severe hypercalcemia can cause permanent renal damage, neurologic dysfunction, coma, and cardiac arrhythmias. Therefore, calcium levels must be closely monitored before, during, and after denosumab administration. 

Unfortunately, transformation of giant cell tumor of bone to malignant osteosarcoma has been observed with treatment with denosumab. This complication should be seriously considered when treating patients with giant cell tumor of bone. Broehm et al reported 2 cases of transformation of recurrent giant cell tumor of bone to osteosarcoma during treatment with denosumab. Santosh et al also reported that a giant cell tumor of bone acquired the osteosarcoma phenotype after exposure to denosumab therapy. Further research is urgently needed investigating the treatment of giant cell tumor of bone. 

On the basis of their clinical experience, Pittman et al suggested general guidelines to prevent and/or manage the side effects of denosumab. Research is needed to investigate the side effects profile of denosumab and to develop effective prevention and management protocols.

CONCLUSION

The RANKL–RANK–OPG pathway is the primary regulator of bone homeostasis. Excessive osteolysis is implicated in the pathophysiology and complications of primary and metastatic bone tumors. Denosumab has been used to prevent osteolysis and to promote bone regeneration when treating giant cell tumor of bone, multiple myeloma, aneurysmal bone cyst, and osteosarcoma. Reduction of the size of unresectable bone tumors is another indication for denosumab. Although studies have reported successful regression of primary bone tumors, especially giant cell tumor of bone, denosumab has been associated with serious side effects even after its discontinuation. Additional clinical studies are needed to establish the use of denosumab as an effective and safe therapy for patients with primary bone tumors.

REFERENCES


