# CASE REPORTS

## Targeting receptor-activator of nuclear kappaB ligand in aneurysmal bone cysts: verification of target and therapeutic response

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**Aneurysmal bone cyst (ABC) is a benign tumor of bone presenting as a cystic, expansile lesion in both the axial and appendicular skeleton. Axial lesions demand special consideration, because treatment-related morbidity can be devastating. In similar lesions, such as giant cell tumor of bone (GCTB), the receptor-activator of nuclear kappaB ligand (RANKL)-receptor-activator of nuclear kappaB (RANK) signaling axis is essential to tumor progression. Although ABC and GCTB are distinct entities, they both contain abundant multinucleated giant cells and are osteolytic characteristically. We hypothesize that ABCs express both RANKL and RANK similarly in a cell-type specific manner, and that targeted RANKL therapy will mitigate ABC tumor progression. Cellular expression of RANKL and RANK was determined in freshly harvested ABC samples using laser confocal microscopy. A consistent cell-type-specific pattern was observed: fibroblastlike stromal cells expressed RANKL strongly whereas monocyte/macrophage precursor and multinucleated giant cells expressed RANK. Relative RANKL expression was determined by quantitative real-time polymerase chain reaction in ABC and GCTB tissue samples; no difference in relative expression was observed ($P > 0.05$). In addition, we review the case of a 5-year-old boy with a large, aggressive sacral ABC. After 3 months of targeted RANKL inhibition with denosumab, magnetic resonance imaging demonstrated tumor shrinkage, bone reconstitution, and healing of a pathologic fracture. Ambulation, and bowel and bladder function were restored at 6 months. Denosumab treatment was well tolerated. Post hoc analysis demonstrated strong RANKL expression in the pretreatment tumor sample. These findings demonstrate that RANKL-RANK signal activation is essential to ABC tumor progression. RANKL-targeted therapy may be an effective alternative to surgery in select ABC presentations. (Translational Research 2014;164:139–148)**

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**Abbreviations:** ABC = aneurysmal bone cyst; GCTB = giant cell tumor of bone; MNGC = osteoclast-like multinucleated giant cell; qRT-PCR = quantitative real-time polymerase chain reaction; RANK = receptor-activator of nuclear kappaB; RANKL = receptor-activator of nuclear kappaB ligand

## AT A GLANCE COMMENTARY

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### Background

Although aneurysmal bone cysts (ABCs) can be managed surgically in most cases, axial-based lesions can be devastating as a result of high recurrence rates and treatment-related morbidity. Given the demonstrated success of denosumab in giant cell tumor of bone, we felt it was critically important to investigate the receptor-activator of nuclear kappaB ligand/receptor-activator of nuclear kappaB signaling axis in ABCs.

### Translational Significance

The patient's dramatic response to targeted therapy not only provides hope for patients with refractory, axial-based ABCs, but also suggests strongly the paradigm that receptor-activator of nuclear kappaB ligand/receptor-activator of nuclear kappaB signaling drives tumorigenesis. These data provide impetus for further study into the role of denosumab in select presentations of ABC.

Aneurysmal bone cyst (ABC) is a benign tumor of bone that presents as a cystic, expansile lesion within the metaphyses of long bones or sites within the axial skeleton. Histologically, ABCs are characterized by blood-filled cavities separated by septae comprised of spindle, inflammatory, and numerous osteoclastlike multinucleated giant cells (MNGCs).[1,2] ABCs occur most frequently in those younger than 21 years,[3,4] but have been reported in subjects beyond the sixth decade of life.[5] They are primary lesions in approximately 70% of cases and are associated frequently with recurrent translocations involving the *USP6* gene, most commonly t(16; 17).[6,7] ABCs are known to arise from primary bone tumors as secondary neoplasms, including giant cell tumor of bone (GCTB), chondroblastoma, fibrous dysplasia, nonossifying fibromas, osteoblastoma, and others.[2]

ABCs constitute approximately 15% of all primary spine tumors[8-10] and are associated with the sacrum in 20%–30% of patients.[3,11,12] Sacral ABCs present unique treatment challenges because of the proximity of adjacent visceral and neurovascular structures, as well as the vascularity of these tumors, which can expose the patient to extreme risk of bleeding.[4,13,14] Reported iatrogenic surgical risks include neurologic injury, and visceral and reproductive organ injury.[4] Overall, the morbidity related to sacral ABC progression and treatment can be substantial.[11]

The receptor-activator of nuclear kappaB ligand (RANKL) cytokine is an essential regulator of osteoclastogenesis.[15,16] RANKL is expressed in osteoblast lineage precursors,[17] cells of hematopoietic lineage,[18] megakaryocytes,[19] and in cellular elements of GCTB.[20-24] On binding of RANKL to its cognate receptor, receptor-activator of nuclear kappaB (RANK), osteoclast differentiation is initiated through activation of nuclear factor of activated T cells, calcineurin-dependent 1 (or NFATc1) in monocyte/macrophage precursors.[25] RANKL is an essential factor in the development and progression of GCTB, and is known to regulate giant cell formation in this tumor.[26,27] As a result, RANKL is primarily responsible for the uncoupling of bone formation that is the hallmark of GCTB, and potentially other giant cell-rich neoplasms. Other benign tumors of bone also contain significant populations of giant cells (eg, ABC, chondroblastoma, nonossifying fibroma, pigmented villonodular synovitis); however, the degree to which osteolysis is dependent on RANKL in each of these disease entities is unclear.[28]

Denosumab (Amgen; Thousand Oaks, Calif) is a human monoclonal antibody that binds to and inhibits RANKL.[29,30] Targeting RANKL with denosumab has proved efficacious as an antiresorptive strategy,[31-36] for reducing the number of skeletal-related events in patients with cancer,[36-41] and for the treatment of refractory or recurrent GCTB.[26,27] RANKL is highly expressed in the stroma of GCTB, and it drives formation and activation of osteoclastlike MNGCs through interaction with RANK on the surface of monocyte or macrophage lineage precursors.[21,23,28,30,42] Although ABC is distinct histologically and molecularly from GCTB,[43] both share a phenotype of aggressive osteolysis and robust MNGC formation. It is not clear whether a similar paradigm of RANK-RANKL axis upregulation is essential to disease progression in ABCs. Data confirm that MNGCs in ABCs express markers typical of osteoclast lineage and are capable of bone resorption.[44,45] RANKL expression has been documented in ABCs and in a variety of other giant cell-rich neoplasms.[28] The observation that RANKL enhances MNGC formation and activation in ABC-derived cd14+ monocytes suggests that, similar to GCTB, this process may also be RANKL dependent.[45]