Original paper

Morphologic evaluation of the effect of denosumab on giant cell tumors of bone and a new grading scheme

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Giant cell tumor (GCT) is a rare, usually benign but locally aggressive neoplasm. Recent studies suggest new approaches in light of the elucidation of molecular pathways in bone. The osteolytic nature of GCT is caused by the receptor for activating nuclear factor-kB ligand (RANKL) associated osteoclasts. Denosumab is a monoclonal antibody that affects GCT through RANKL and it prevents normal and neoplastic osteolysis. The aim of this study is to evaluate the histopathologic alterations due to denosumab treatment and the efficiency of this drug in GCT therapy.

Ten patients had been treated with denosumab and were included in the study. Pretreatment biopsies were interpreted as conventional GCTs and posttreatment biopsies of the ten patients' GCTs were classified in accordance with the grading system. Only one patient had tumor remaining after treatment.

There is limited data on histopathologic alterations that follow denosumab treatment. The bone pathologist should keep these changes in mind because they mimic different types of bone tumors. Furthermore, there is no widely accepted grading system to evaluate the effect of denosumab in GCT. Our study suggested a scheme that would be helpful to evaluate the efficiency of denosumab treatment in GCT.

Key words: denosumab, giant cell tumor of bone, pathology, grading, treatment, RANKL.

Introduction

Giant cell tumors (GCTs) are rare, usually benign, locally-aggressive neoplasms that account for 5% of all primary bone tumors. Although their behavior is benign in most cases, they may progressively enlarge and unexpectedly extend through the bone cortex into the surrounding soft tissues. Histopathologically, GCTs comprise proliferating mononuclear rounded stromal cells, osteoclastic giant cells, and mononuclear histiocytic cells [1]. Local recurrence and metastasis can still occur regardless of standard treatment procedures, which include intralesional curettage, high speed burring, application of local adjuvants and defect-filling with bone cement or grafting. Recent studies have suggested new approaches in light of the elucidation of molecular pathways in bone [2]. The receptor for activating nuclear factor-kB ligand (RANKL), one of the molecules of bone metabolism, interacts with RANK, which is expressed in the osteoclast membrane [3, 4]. This interaction is substantial and promotes differentiation, formation, and survival of osteoclasts [5]. The osteolytic nature of GCT is caused by RANKL-associated osteoclasts. Denosumab is a monoclonal antibody that affects GCT through RANKL and prevents normal and neoplastic osteolysis [6].

The aim of this study is to evaluate histopathologic alterations of GCT after denosumab treatment,

and to form a new histologic classification system for tumor response.

Material and methods

This study was conducted with the permission of the ethical review board from the Ministry of Health of Turkey for each patient who was treated with denosumab. Ten patients with GCT of the bone were enrolled in the study. Each patient underwent an initial Jamshidi biopsy, which was evaluated before treatment by Cukurova University Department of Pathology. One hundred twenty milligrams of denosumab (Xgeva) was given on day 0, 8, and 15, followed by monthly subcutaneous injections. An average of 7.6 cycles of treatment was given according to the availability of the drug and ossification of the tumor. After therapy completion, 7 patients underwent curettage followed by high-speed burring, adjuvant phenol, and grafting or cementation, and 3 patients underwent wide resection. Demographic data, localization of the tumor, and the total number of denosumab cycles are shown in Table I. The initial and subsequent pathology samples were evaluated in our institution. Initial and latter biopsies were decalcified and fixed in 10% buffered formalin at room temperature, dehydrated, and paraffin embedded in overnight processing. Four-micrometer-thick sections were routinely stained with hematoxylin and eosin and examined under a light microscope by 2 pathologists (one of whom was experienced in bone pathology) and graded according to the relative amounts of inflammation, fibrous tissue, woven bone, precancellous bone and cancellous bone. Clinicopathologic features such as age, gender, and tumor location were reviewed with histopathologic findings of pre- and posttreatment specimens.

The tumors were graded as follows:

- Grade 1: Inflammation, fibrous tissue and/or woven bone,
- Grade 2: Woven bone and precancellous, cancellous bone.

Among ten patients, eight patients'pretreatment and posttreatment specimens and remaining two pa-

Table I. Summary of ten GCT cases

Age/Sex/Tumor Location	Initial Biopsy	Treatment Cycle	Histopathologic Grade
Patient 1 54 years-old, F left tibia	GCT	13	1
Patient 2 39 years-old, M left femur	Areas of foci resembling GCT	9	1
Patient 3 35 years-old, F right femur	GCT	10	1
Patient 4 29 years-old, F left fibula	GCT; (brisk mitotic figures necrosis) + secondary aneurysmal bone cyst	10	2
Patient 5 39 years-old, M right humerus	GCT	6	2
Patient 6 31 years-old, F sacrum	GCT	4	2
Patient 7 27 years-old, M right femur	GCT	6	1
Patient 8 21 years-old, F left femur	GCT	6	2
Patient 9 44 years-old, M right radius	GCT	6	2
Patient 10 37 years-old, F left radius	GCT	6	2

tients' posttreatment specimens were evaluable for RANKL expression by immunohistochemically. Formalin fixed, paraffin embedded tissue sections were incubated overnight, deparaffinized, hydrated and the sections were heated for 20 minutes at 121°C in antigen retrieval buffer, blocked against endogenous peroxidase activity and staining reagent. Immunohistochemical analysis was carried out using automated staining methods (Benchmark XT Autostainer; Ventana; Roche). Tissue sections were blocked against endogenous proteins and staining reagents. Tissue sections were stained with a rabbit anti-human RANKL antibody (ab9957; Abcam, Cambridge, UK).

Results

In total, 10 patients had been treated with denosumab and were included in the study, of which 6 were women and 4 were men. The mean age was 35.6 years (range, 21-54 years). Five of the ten patients' tumors were located in the region of the knees (3 distal femur, 1 proximal tibia, and 1 proximal fibula); one was in the proximal femur; one was in the proximal humerus; two were in the distal radius; and one was in the sacrum. Pretreatment biopsies were interpreted as conventional GCTs composed of round-oval mononuclear neoplastic cells and osteoclast-like giant cells (Fig. 1). In addition to this conventional morphology, the second and fifth patients' initial biopsies had foci of osteoclastic giant cells intermingled with large areas of histiocytic cells, lymphocytes, and hemorrhage; the fourth patient's first biopsy presented with brisk mitotic activity with areas of necrosis of GCT and a secondary aneurysmal bone cyst. Posttreatment biopsies were evaluated according to inflammation, fibrosis, osteoid formation. There was only one case who had tumor remaining after treatment (Fig. 2). The first case's posttreatment material was composed of inflammatory, fibrohistiocytic, hemorrhagic and xanthomatous areas intermingled with anastomosing woven bone. The histologic picture was reminded of fibrous dysplasia, nonossifying fibroma. The second case's posttreatment material was composed of heavily collagenous matrix and inflammatory reaction. The parallel collagen fibers and inflammatory cells and mononuclear cells mimic intraosseous schwannoma. The third case's posttreatment material was composed of dense mononuclear inflammatory cells intermingled with woven bone. The following three cases were similar to osteoblastoma that were composed of anastomosing woven and precancellous bone and loose vascular connective tissue, hemosiderin laden macrophages. The seventh case's posttreatment material was composed of mildly cellular fibroblastic proliferation resembling desmoplastic fibroma of bone. The following case had areas of bone formation and resorbtion, fibrosis and hemorrhage resembling fracture healing. The last two cases had areas of precancellous bone formation and loose vascular connective tissue. The histopathologic alterations of posttreatment specimens include nonossifying fibroma, aneurysmal bone cyst, desmoplastic fibroma (Fig. 3), osteoblastoma, nidus formation (Fig. 4), fibrohistiocytic lesions (Fig. 5), hemangioma (Fig. 6) or schwannoma (Fig. 7). The ten patients' GCTs were classified in accordance with the grading system defined above (Table I). The treatment cycles ranged between 4-13 cycles.

Pretreatment tumor samples of eight patients were composed of cellular RANKL positive uniformly distributed tumor stromal cells (Fig. 8). Posttreatment tumor samples of all of ten patients were variably expressed RANKL positive stromal cells in addition to marked reduction of osteoclast-like giant cells in the samples (Fig. 9).

The reduction of expression of RANKL was detected in case number 1, 4, 6 and 10 after treatment.

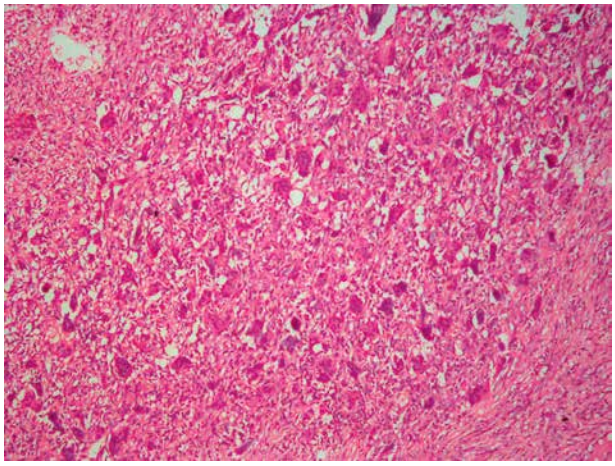

Fig. 1. Conventional GCT (HE, original magnification 100×)

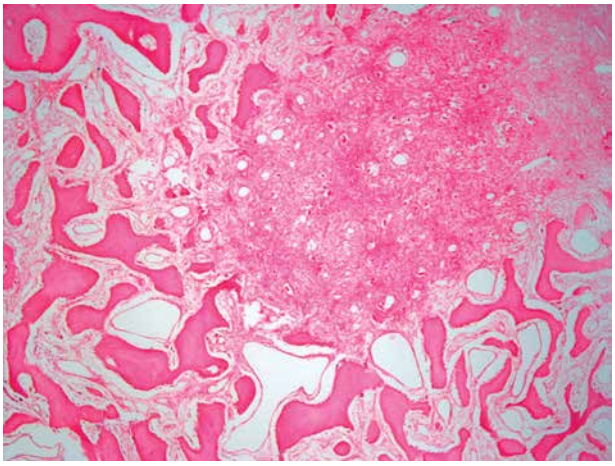

Fig. 2. Remaining GCT is seen in the center of precancellous bone formation. Grade 2 (HE, original magnification 40×)

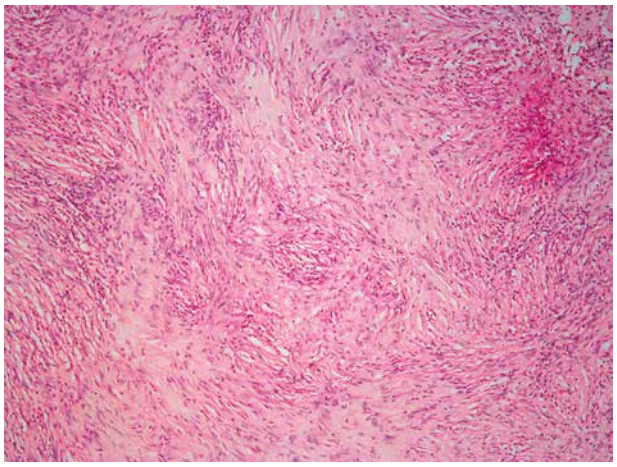

Fig. 3. Bundles of spindle cells with abundant collagenous matrix lacking osteoid resembles desmoplastic fibroma of bone. Grade 1 (HE, original magnification 100×)

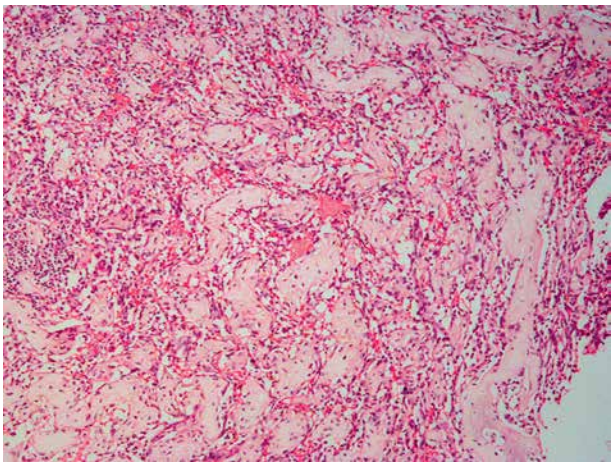

Fig. 4. Anastomosing pattern of reactive bone formation with osteoblastic rimming mimics nidus tissue. Grade 2 (HE, original magnification 100×)

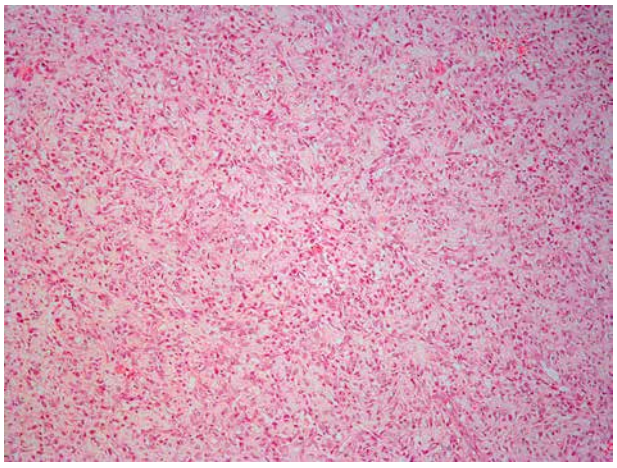

Fig. 5. Fibrohistiocytic areas are composed of mononuclear cells lacking osteoid formation. Grade 1 (HE, original magnification 40×)

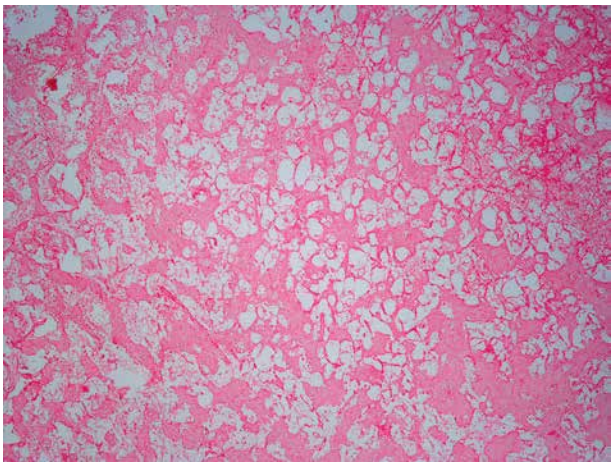

Fig. 6. The immature woven bone interdissecting with pseudoangiomatous spaces resembling intraosseous hemangioma or lymphangioma. Grade 2 (HE, original magnification 40×)

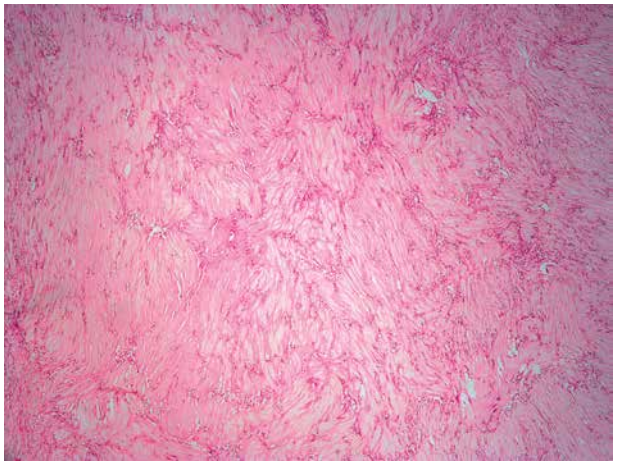

Fig. 7. The parallely arranged collagenous fibers and scattered mononuclear cells resemble intraosseous schwannoma. Grade 1 (HE, original magnification 40×)

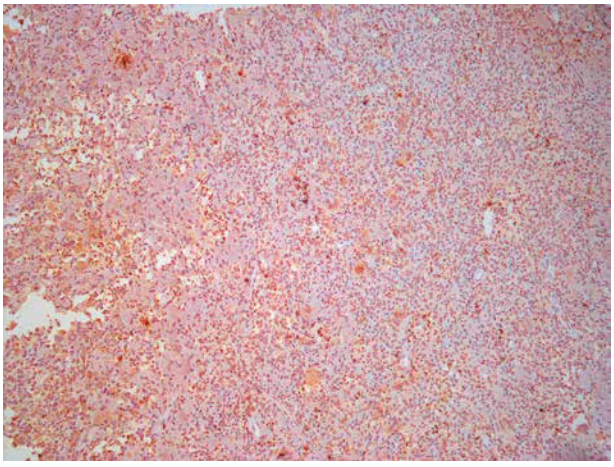

Fig. 8. RANKL expression in tumor stromal cells by IHC in pretreatment sample (Case number 1, original magnification 40×)

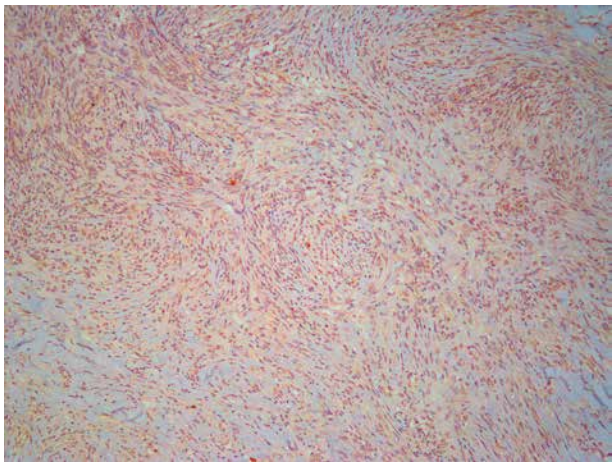

Fig. 9. Reduced expression of RANKL in tumor stromal cells by IHC in posttreatment sample (Case number 1, original magnification 40×)

The expression of RANKL was not vary significantly in posttreatment sample in case number 2, 3, 5 and 9 after treatment. Remaining two cases' pretreatment samples were unadequate for immunohistochemical staining but posttreatment samples showed RANKL expression in tumor stromal cells.

Discussion

Giant cell tumors are well-defined benign neoplasms of unpredictable course due to recurrence and metastasis. Surgery is the preferred treatment but treatment with denosumab was recently shown to delay the need for surgery and prevent morbid surgical procedures [7].

Bone remodeling is a continuous process that involves osteoclasts and osteoblasts [8, 9]. The molecular biology of bone forming is well defined and includes a variety of molecular pathways and molecules [10]. Osteoprotegerin (OPG) is a soluble RANKL-binding protein that binds RANKL. This molecule prevents RANKL from combining with RANK on the osteoclast membrane. Denosumab mimics endogenous OPG and prevents RANKL from binding with RANK, which leads to inhibition of osteoclastic differentiation, activation, and bone resorption [5, 11].

There is limited data on histopathologic alterations that follow denosumab treatment. Single case reports indicated alterations in morphology such as benign fibrous histiocytoma-like (BFH) lesions [12]; fibrous cells with partial reactive bone formation; bone regeneration; aggregated inflammatory cells [13]; distinctive pseudosarcomatous spindle cell proliferation with matrix production and paucity of giant cells and mitosis; mimicking osteogenic sarcoma [14]; reactive stromal cells and scattered spindle cells with elongated oval-shaped nuclei without evident atypia and diffusely clustered foamy macrophages [15]; 5-10% necrosis with remaining GCT [16]; extensive necrosis and fibrosis without GCT [17]; lacking giant cells in the metastatic GCT to lung [18]. Branstetter *et al.* evaluated GCTs' pre and posttreatment specimens by immunostaining with RANK, RANKL [19]. They demonstrated the osteoclastic arrest and presented histopathologic and immunohistochemical findings but they did not classify these changes. Girolami *et al.* evaluated GCTs' pre- and posttreatment specimens by molecular analysis in addition to immunohistochemistry [20]. They found that denosumab changes the microenvironment of the tumor cells instead of eliminating them. Moreover, the inhibition of recruitment of osteoclast like giant cells, significant reduction of proliferative activity and microvessel density are found to correlate treatment efficiency. In the present series of GCT, we observed permanently expression of RANKL among tumor stromal cells in four cases and reduction of RANKL expression in four cases at pre and posttreatment samples.

We also tried to succinctly describe the morphologic appearances in a grading system for simplification. Posttreatment histopathology of denosumab includes reactive and neoplastic lesions, areas of foamy macrophages, cystic hemorrhage, hemosiderin-laden macrophages, scattered giant cells, fibrous tissue, woven bone that resemble aneurysmal bone cysts. Similar findings may be found in other conditions and these should not be confused with those of GCTs, examples include pigmented villonodular tenosynovitis, osteosarcoma, fibrous dysplasia, benign fibrous histiocytoma, osteoblastoma. The pathologist must be aware of therapy and physicians should inform pathologists to avoid misdiagnosis. Like many other studies, this study also suggests that denosumab treatment may cause morphologic alterations due to osteoclastic arrest. It remains a matter of debate as to whether these histopathologic features relate to treatment dose, and also whether and when the histopathologic features relapse after treatment cessation. Further studies are needed to answer these questions. The case number is the limiting factor in our study; however, we were able to determine that at least 4 doses must be applied to achieve a good response. This grading scheme may be used in the field of therapy response to denosumab for example; good response represents increased grade.

In conclusion, the morphologic alterations after denosumab treatment can be variable and mimic a variety of benign and malignant lesions. The pathologists must keep these deceptive lesions in mind, which may result from denosumab therapy. Furthermore, there is no widely accepted grading system to evaluate the effect of denosumab in GCTs. This study is the only one to summarize the suggested

histopathologic changes after therapy with a grading scheme.

We would like to thank David F. Chapman, BSc for language editing of the manuscript.

The authors declare no conflict of interest.

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