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Clinical Trial

Efficacy of denosumab in joint preservation for patients with giant cell tumour of the bone

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Abstract ***Background:*** Giant cell tumour of the bone (GCTB) is an aggressive osteolytic primary tumour. GCTB is rich in osteoclast-like giant cells and contains mononuclear cells that express RANK ligand (RANKL), a key mediator of osteoclast activation. The potential therapeutic effect of denosumab was investigated with special reference to its role in joint preservation.

Methods: In this prospective non-randomised study patients with GCTB received denosumab for 6–11 months preoperatively. Serial radiographs and biopsy and resection tumour specimens were used to monitor response to denosumab.

Results: All 20 patients experienced pain relief in the first month of treatment. All patients demonstrated a positive radiographic response with improved subchondral and cortical bone which allowed intralesional tumour resection and preservation of the joint and articular surface in 18 cases. Histological examination following denosumab revealed rarely detectable osteoclast-like giant cells. There was an obvious increase in osteoid matrix and woven bone which showed rare RANK staining amongst the mononuclear cells and only focal RANKL positivity. At median 30 months follow-up after resection, local tumour recurrence occurred in three patients.

Conclusion: Denosumab provides favourable and consistent clinical, radiographic and pathologic responses which facilitates less aggressive surgical treatment, especially joint

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preservation. However, the local recurrence rate for GCTB following resection does not seem to be affected by denosumab and remains a concern.

1. Introduction

Giant cell tumour of the bone (GCTB) is a rare, locally aggressive osteolytic skeletal neoplasm that most commonly occurs in young adults [1,2]. Although generally considered to be benign, rarely it can metastasise despite maintaining a benign histology. Even more uncommonly, GCTB has the potential to transform into a true malignant tumour [3]. However, it is generally treated as a locally destructive bone tumour which causes pain, limitation of joint motion and impaired function and is at risk for intra-articular and periarticular pathologic fracture given that it occurs in epiphyseal-metaphyseal locations.

GCTB is characterised by the distribution of numerous osteoclast-like giant cells amongst a mononuclear cell background [4]. The giant cells tend to be larger, and include a greater number of nuclei, than typical osteoclasts. The mononuclear component consists of two sub-populations, which are difficult to differentiate morphologically: macrophage-like cells and neoplastic mesenchymal stromal cells. The neoplastic stromal cells tend to be spindle shaped, while the macrophage-like cells are more ovoid-round in morphology. Both the macrophage- and osteoclast-like cell populations express receptor activator of nuclear factor kappa B (RANK), while the stromal cells express RANK ligand (RANKL) [5,6]. In the appropriate milieu [7], the stromal cells induce the recruitment and fusion of macrophage-like cells into osteoclast-like cells in a RANK/RANKL dependent manner [4]. Recently mutations in H3F3A were identified as the driver mutations in a majority of GCTB [8]. It is ultimately the osteoclast-like giant cells that are responsible for the locally aggressive osteolytic activity of the tumour [5]. On the basis of histological findings, however, the clinical course of the disease is not predictable [2].

Surgical therapy remains the mainstay of treatment for GCTB but has been associated with high rates of local tumour recurrence and significant complications [9–11]. Decision making regarding the surgical approach must balance the morbidity of treatment against the likelihood of local tumour recurrence. The therapy of choice is extended intralesional curettage, augmented with high-speed burring of the tumour cavity, in order to maintain anatomic integrity of the involved bone and especially the articular surface, even in the setting of a pathologic fracture [12–14]. En bloc resection of the involved bone typically lowers the risk of local recurrence but often necessitates prosthetic joint reconstruction which can be problematic in young adults especially with long term followup. Adjuvant treatment has been advocated in an attempt to reduce the rate of local recurrence, which can be as high as 50% [10]. A wide variety of adjuvant options are commonly used, including preoperative embolisation, water, hydrogen peroxide, phenol, cryotherapy, argon beam coagulation and bone cement [1,12,15,16], however the efficacy of most remains unproven [10,17–19].

Denosumab is a fully humanised monoclonal antibody that specifically binds and inhibits RANKL activation of RANK [20], thereby inhibiting osteoclastogenesis and osteoclast-mediated bone destruction. In patients with multiple myeloma or bone metastases from breast or prostate cancer, subcutaneous administration of denosumab provides rapid and sustained suppression of bone turnover and decreases pain and other skeletal-related events [21,22]. In an ongoing open-label, phase II study, the safety and efficacy of denosumab in more than 280 patients with complicated GCTB was reported [23–26]. On the basis of these data, the food and drug administration (FDA) approved denosumab for the treatment of patients with unresectable GCTB, or when surgery is likely to result in severe morbidity. These initial studies were focused on safety and efficacy with primary end-points being the type, frequency, and severity of adverse events, and a secondary end-point of time to disease progression. The authors concluded that denosumab inhibits osteoclastogenesis in GCTB and may prevent, or at least postpone surgical procedures, since 74% of the patients who originally were planned for surgery did not undergo the procedure. The median follow-up of patients classified as 'salvageable' was only 9.2 months. The downstaging capacity of denosumab was recently described in an interim analysis of 222 patients from a phase II trial [26]. At the data cutoff date, the median duration of denosumab treatment for surgically treated patients was 14.2 (12.0–17.7) months and 84 of these patients (72%) underwent a less morbid procedure than originally planned. In addition, 106 patients had not undergone any surgery and remained on monthly denosumab for a median of 19.5 (12.4–28.6) months. Histopathological findings of GCTB following denosumab treatment were described in only a small follow-up study, and showed evidence of a decrease in both giant cells and tumour stromal cells, as well as an increase in new bone formation [24].

In the present study, we review 20 patients with GCTB in which neoadjuvant denosumab was administered with the goal of decreasing the extent of surgery and subsequent surgical morbidity, and particularly to facilitate joint preservation. The focus of this study was to evaluate the clinical, histological and radiological effects of denosumab treatment in patients with GCTB with special reference to its role in facilitating joint preservation.

2. Materials and methods

Twenty patients with histologically confirmed GCTB (see Table 1 for clinical details) were consecutively enrolled in this prospective clinical study between April, 2011 and November, 2013 to determine if preoperative treatment with denosumab would facilitate surgical resection and reconstruction of GCTB, and in particular joint salvage. All 20 patients were designated as 'high risk' based on associated extensive periarticular bone loss, soft tissue mass and/or pathologic fracture, such that joint salvage was either not possible or questionable at presentation, and the planned surgical procedures would be associated with potentially poor function and significant morbidity. The first 12 patients with GCTB were treated as part of an Amgen initiated open-label phase II trial of denosumab (ClinicalTrials.gov: NCT00680992)[23,25], and the final eight patients received similar treatment after that study was closed to enrolment. All patients were skeletally mature and had either newly diagnosed (n = 17) or recurrent GCTB (n = 3). Exclusion criteria included a history of malignancy within the past 5 years, radiation to the affected region, previous treatment with bisphosphonates or denosumab, prior history or current evidence of osteonecrosis or osteomyelitis of the jaw, an active dental or jaw condition requiring oral surgery, evidence of non-healed dental or oral surgery and pregnancy. All patients gave written consent for this study which was approved by the Research Ethics Board.

Table 1
Demographic/clinical data and radiological response to denosumab.

	N = 20
Female (%)	10 (50%)
Median age, years (range)	28 (18–64)
Median follow-up, month (range)	30 (20–45)
Location	
Ankle/foot	3
Knee	12
Wrist	3
Shoulder	1
Spine	1
Previous surgery	
None	17
1	1
>1	2
Clinical findings at diagnosis	
Pathological fracture	6
Pain	20
Swelling	16
Decreased ROM	16
Campanacci classification	
Stage II	7
Stage III	13
Improvement of cortical and subchondral bone thickness	N = 20
None	2
Minor	3
Significant	15
Mineralised sclerotic rim circumference	
≥95%	13
≥80% ≤ 95%	5
n/a	2
Summary of radiographic response	
Complete response	0
Partial response	20
Stable disease	0
Progressive disease	0

n/a, not applicable; ROM, range of motion.

At presentation, all patients had localised pain and tenderness to palpation at the site of the lesion. A visible or palpable mass was detected clinically in 16 patients. Sixteen patients had decreased range of motion (ROM), activity-related pain and functional limitations. Six patients presented with a pathological fracture. Pulmonary metastasis was not identified in any of the patients at the time of diagnosis. In the three patients who presented with confirmed recurrent GCTB at enrolment, we used the radiographs obtained immediately prior to neoadjuvant denosumab treatment as our reference, to avoid conflict of assessment of response.

All 20 patients received subcutaneous denosumab (120 mg) every 4 weeks with additional doses on days 8 and 15 of the first cycle of treatment. Patients were planned to receive a minimum of six cycles of denosumab prior to surgery, unless there was still evidence of ongoing clinical and radiographic improvement. No patient received additional denosumab treatment following definitive surgery. All patients took daily supplements of calcium (≥500 mg) and vitamin D (≥400 IU) supplements. Adverse events were recorded and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 [27].

2.1. Radiological evaluation

The skeletal lesions were categorised, according to the system of Campanacci [28], as grade I (quiescent), grade II (active), or grade III (aggressive) based on the initial plain radiograph and computed tomography (CT) scans. Following denosumab treatment and prior to definitive surgery, the region of interest was re-evaluated using plain radiographs and MRI or CT scans as appropriate. The pre- and post-treatment images were compared by two musculoskeletal radiologists. We developed the following objective radiographic tumour response criteria: size of the lesion, extent of sclerosis

and bone formation within the lesion, percentage of circumferential mineralised sclerotic rim surrounding the lesion, and cortical and subchondral bone thickness. The radiographic results were summarised in four categories: complete response, partial response, stable disease, or progressive disease.

2.2. Surgical management

Surgery was planned following completion of preoperative denosumab, usually 1 month following the final drug injection. All GCTB lesions were managed as described previously [12,15] with extensive curettage through a large cortical window to remove all visible tumour, followed by use of a high speed burr along the periphery of the lesion, lavage with peroxide and pulsatile lavage with sterile water [12,13,15]. Joint preservation was the main objective of surgical treatment, therefore, whenever possible reconstruction of the osseous defect was performed using bone allograft +/− internal fixation rather than arthroplasty or resection. If the articular surface could not be salvaged, then we attempted to reconstruct the joint using a conventional arthroplasty rather than with a tumour endoprosthesis if possible.

2.3. Histological evaluation

Tumour samples, from both the diagnostic biopsy and post-denosumab curettage procedures were analysed and compared histologically using standard methods. In many cases, mild decalcification was required following tissue fixation. All samples were stained by routine haematoxylin and eosin to permit morphologic assessment of the specimens. Representative sections from both the pre- and post-treated tumours were selected for immunohistochemical evaluation using antibodies targeting RANK and RANKL. Briefly, slides were dried overnight, deparaffinised, and hydrated. Heat induced epitope retrieval was performed with a MicroMed T/T Mega microwave (Milestone, Sorisole, Italy) using TRIS buffer at 115 °C for 3 min. Staining was performed using an automated stainer (Dako North America Inc, Carpinteria, CA, United States of America [USA]). The primary antibodies were RANK (CedarLane; Clone 9A725; 1:500) and RANKL (CedarLane; Clone 12A668; 1:100). Staining was visualised using the MACH 4 universal detection system (Biocare Medical, Concord, CA, USA).

2.4. Patient follow-up and functional outcome

After the initial postoperative visit, patients were followed every 3 months for the first 2 years and then every 6 months thereafter. Routine follow-up included clinical examination and conventional radiography of the operative site. Chest radiographs were performed annually. CT and magnetic resonance imaging were used for further investigation when plain radiographs demonstrated a suspected relapse (e.g. bone resorption, expansile change, soft tissue swelling) or when clinical signs and symptoms suggested recurrence, despite negative radiographs. Patient function was assessed using the musculoskeletal tumour society rating scales (MSTS) [29] and the Toronto extremity salvage score (TESS) [30]. The MSTS 87 (1987 version) and MSTS 93 (1993 version) are clinician-rated measures of impairment which are joint or limb specific, respectively, while the TESS is a patient-completed measure of physical disability [31], reflecting patient's ratings of the difficulty experienced in performing routine daily activities. The MSTS-87 rating scale ranges from 0–35 and both the MSTS-93 and TESS range from 0–100. For each measure, higher scores reflect better function. Functional outcome scores were compared using the Wilcoxon rank-sum test.

2.5. Statistical analysis

Categorical and ordinal data are described with use of frequencies. Patient age and the follow-up times are expressed as means and standard deviations together with the minimum and maximum values. The hazard ratio for a local recurrence was analysed with use of multivariate Cox regression for clinical and radiographic factors at tumour presentation. The significance level was set at 0.05 for all statistical tests.

3. Results

All 20 patients completed the treatment protocol and were available for follow-up after preoperative denosumab, tumour resection and osseous reconstruction (Table 1). The patients received an average of 8.8 doses of denosumab preoperatively (median 8, range 8–13): 14 patients received eight doses (i.e. six cycles), while the remaining six patients received up to five extra injections. No patients received denosumab following definitive surgery. During the first month of treatment, all patients reported a significant decrease in pain and after 6 months, 16 of 20 patients reported being pain-free. Following denosumab treatment most patients were also found to have improved joint ROM and better overall function, although the presence of a soft tissue mass did not typically resolve. During the denosumab treatment period, six patients (30%) reported grade I or II adverse events (fatigue, headache, nausea, arthralgia), while two patients had transient hypocalcaemia that was treated with increased oral calcium and vitamin D intake. No severe adverse events were reported.

The radiographs obtained immediately prior to commencing denosumab treatment demonstrated the typical features of GCTB including osteolysis, bubble-like multilocular appearance with thinned and expanded

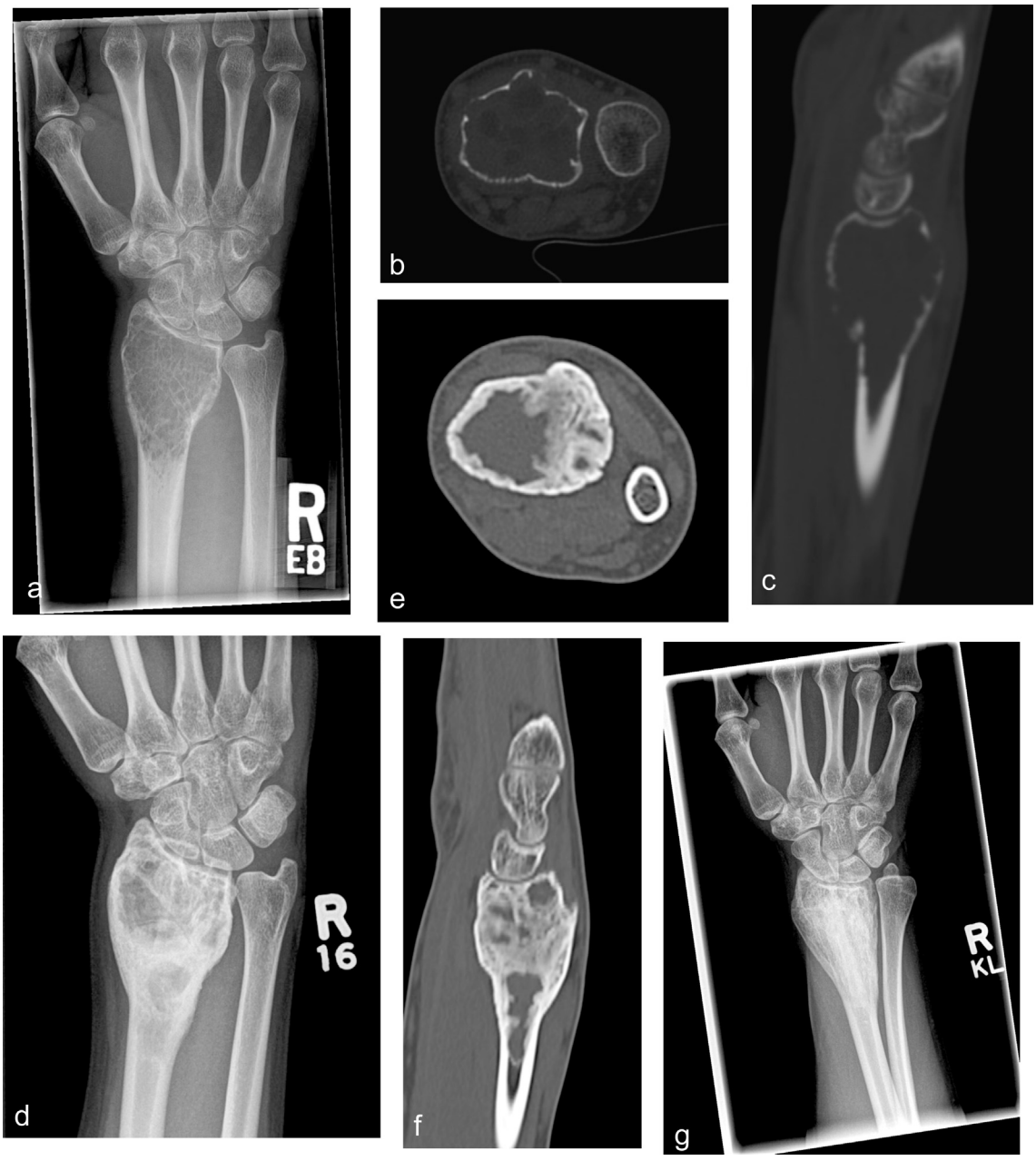

Fig. 1. A 22-year-old female with a distal radius GCTB. a. Plain radiograph of a GCTB of the distal radius demonstrating a peri-articular lytic lesion with involvement of the articular surface and cortical bone. b and c. CT (axial and sagittal) images demonstrating expansion of the distal radius with cortical and subchondral thinning as well as cortical discontinuity. d–f. Plain radiograph (d) and CT images (axial and sagittal; e, f) obtained following denosumab treatment demonstrate significant cortical and subchondral thickening, reconstitution of an intact cortex circumferentially, and central sclerosis. g. Plain radiograph following a 'joint-salvage' procedure with allograft bone reconstruction. The articular surface has been preserved. GCTB, giant cell tumour of the bone; CT, computed tomography.

cortical bone in epiphyseal/metaphyseal regions (Figs. 1a–c and 2a–c). In six patients, a pathological fracture was clearly identified, while five patients demonstrated areas of full thickness cortical bone defects on plain radiographs and CT scans. The Campanacci classification system, which describes the local extent of the tumour radiographically, identified seven grade II and 13 grade III lesions. Based on the initial radiological imaging, all 20 patients were classified as being 'high risk' because a joint or bone preserving procedure was considered to be technically challenging which lead to enrolment in this clinical study assessing the effect of preoperative treatment with denosumab on joint preservation.

In 11 patients, there was a mild (10–25%) increase in the size of the lytic GCTB lesion over the course of denosumab (Fig. 3). In five patients, the tumour size remained relatively stable, while four patients

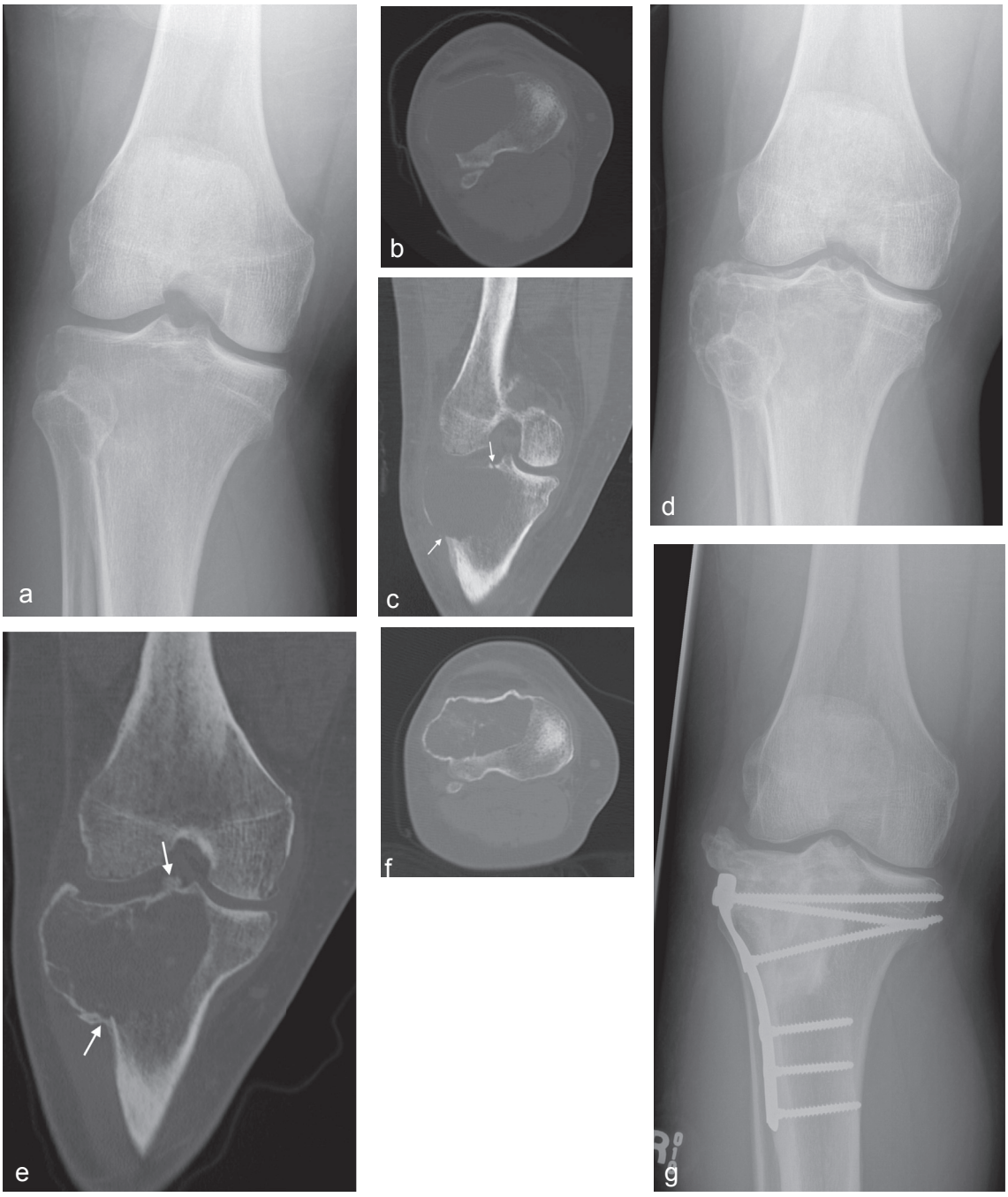

Fig. 2. A 39-year-old male with a proximal tibia GCTB with a pathologic fracture. a–c. Initial plain radiograph (a) and CT images (axial and coronal; b and c) demonstrate the expansile, lytic lesion of a GCTB involving the proximal lateral tibia plateau with significant cortical and subchondral thinning, cortical discontinuity and a pathologic fracture (arrows). d–f. Plain radiograph (d) and CT images (axial and coronal; e and f) following treatment with denosumab demonstrate thickening of the cortex and subchondral bone, re-establishment of cortical continuity and healing of the pathologic fracture (arrows) and mineralisation within the lesion. g. Plain radiograph shows the result following tumour resection and allograft reconstruction with lateral buttress plating. The articular surface has been preserved. GCTB, giant cell tumour of the bone; CT, computed tomography.

demonstrated a reduction in size during treatment. Central sclerosis and new bone formation were detected in all cases, with measurements ranging from 5–70%. In 18 patients, variable improvement of the cortical thickness as well as subchondral bone thickness was identified, and a complete or close to complete mineralised sclerotic rim surrounding the lesion was seen in 13 patients following treatment (Table 1; Figs. 1d–f and 2d–f). No lesion progressed during treatment with denosumab. All six pathological fractures identified at the start of the study demonstrated complete healing during the course of medical treatment. In summary, a partial radiographic tumour response to denosumab was noted in all patients.

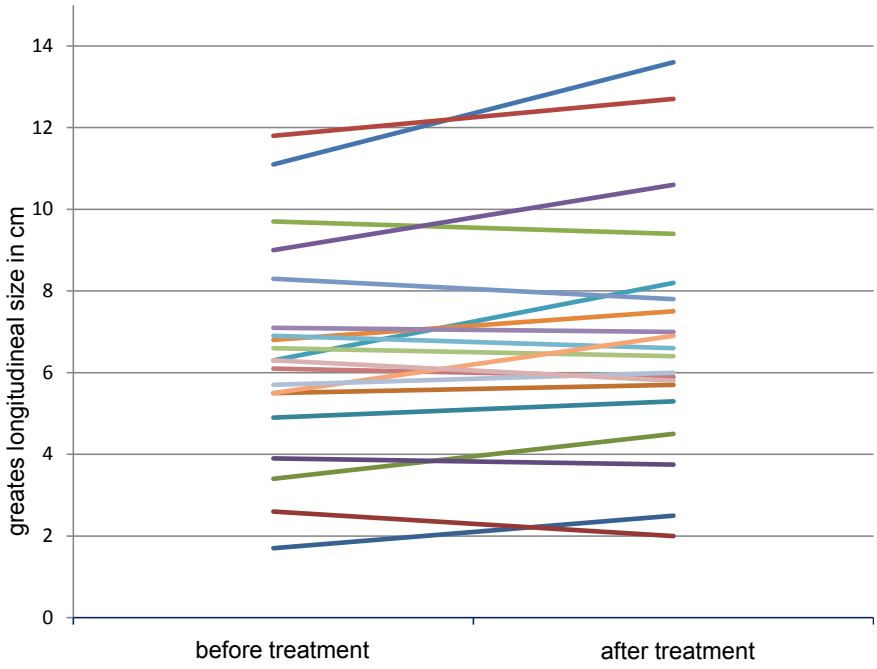

Fig. 3. Change of the greatest longitudinal dimension of the lesion before and after denosumab treatment.

Following preoperative denosumab treatment, no patient required en-bloc resection of the tumour or reconstruction with a tumour endoprosthesis. All 20 patients underwent intralesional resection of the tumour preserving the anatomy of the involved bone, and in 18 cases the joint and articular surface were spared. The cases which initially demonstrated expanded and thinned cortical bone at diagnosis, which could be easily perforated with minimal pressure, were found to have thicker and better quality bone at surgical resection, in keeping with the previously described radiographic response to denosumab. In these cases, a burr or osteotome was typically required to open a window in the bone to access the intramedullary tumour. Even cases which presented with a soft tissue mass were found to have developed an intact layer of bone covering the mass following denosumab treatment. Within the bone, the typical friable giant cell tumour tissue was replaced with gritty, fibrous-like tissue and distinct areas of new bone, which made it difficult to determine when resection of all the tumour tissue was complete. As a result, intra-operative fluoroscopy was often utilised to help guide the extent of tumour resection. The bone defects following tumour resection were reconstructed with allograft in 19 cases, with or without supplemental internal fixation (Figs. 1g and 2g). None of the patients required autogenous bone graft in addition to the allograft.

Joint preservation was not possible in only 2 of 20 (10%) patients. One patient with a pathologic fracture of the proximal tibia associated with extensive cortical bone loss at diagnosis developed re-ossification of the cortex following denosumab such that intralesional tumour resection was possible instead of en bloc resection of the involved bone. However this patient had not demonstrated the typical improvements in pain, knee ROM and function following treatment and was found to have disruption of the joint surface and secondary arthritis at the time of surgery. A knee joint replacement was required, but instead of using a tumour endoprosthesis, a conventional knee arthroplasty was performed and augmented with tantalum cones to replace the area of bone loss, which allowed for early ambulation and physiotherapy. The other patient had GCTB involving the L4 vertebral body associated with a pathologic fracture of the superior and inferior endplates at diagnosis. Despite the positive clinical and radiological improvement following denosumab therapy, an L3-5 spinal fusion with structural allograft and internal fixation was necessary. However, instead of en bloc resection of the L4 vertebral body, the lesser procedure of curettage following denosumab was possible. No postoperative complications were observed. Functional outcomes improved following denosumab treatment and surgical reconstruction as follows: mean TESS changed from 74 at diagnosis to 91 at latest follow-up ($p = 0.039$); mean MSTS-87 improved from 29–32 ($p = 0.065$); and mean MSTS-93 improved from 86–92 ($p = 0.37$).

The median follow-up time after surgical resection was 30 months (20–45 months). Local tumour recurrence occurred in 3/20 patients (15%) at 10, 12 and 25 months after intralesional curettage. None of these patients had a previous GCTB recurrence. We performed multivariate analysis using clinical, radiographic and histologic factors but were unable to identify any

parameter which increased the risk of tumour recurrence. In two patients, repeat intralesional curettage was performed for lesions in the distal femur and proximal tibia, again preserving the anatomy of the involved bone and joint surface. One patient presented with local recurrence in the distal ulna associated with extensive bone destruction and a large soft tissue mass. Since this bone is considered to be 'expendable', locally curative treatment involved en-bloc resection of the distal ulna and soft tissue reconstruction. This patient also developed multiple lung metastases 4 months following distal ulna resection and after a percutaneous needle biopsy of the lung confirmed benign metastasising giant cell tumour, denosumab was restarted.

Histopathological examination of the diagnostic tumour biopsies confirmed a morphology typical of GCTB in all cases (Fig. 4); each case was characterised by sheets of fairly evenly distributed osteoclast-like giant cells admixed with a background of mononucleated cells. There was no significant evidence of aneurysmal bone cyst formation, so-called 'malignant' osteoid or chondroid matrix. Most of the tumours exhibited brisk mitotic activity, but atypical mitotic figures were not identified. Necrosis was rarely present, and was typically punctate. The post-denosumab treated samples were characterised by an absence of osteoclast-like giant cells in all but a few examples; in these cases, the osteoclast-like cells were rare and difficult to identify. The presence of residual giant cells after denosumab treatment did not appear to be associated with local recurrence of GCTB which occurred in three patients, as none of these three individuals were found to have any residual giant cells in their resected tumours. Following denosumab treatment, the residual stroma was generally fibro-osseous, with a zonal pattern. In areas, there were sheets of sclerotic and mineralised woven bone housing osteocytes. Elsewhere there were nodules and interweaving fascicles of spindle cells with bland ovoid nuclei (Fig. 5a). In these areas, variable amounts of collagenous stroma were present between the cells (Fig. 5b and c.) Areas of necrosis, foamy histiocytes and scattered lymphocytes were also occasionally identified.

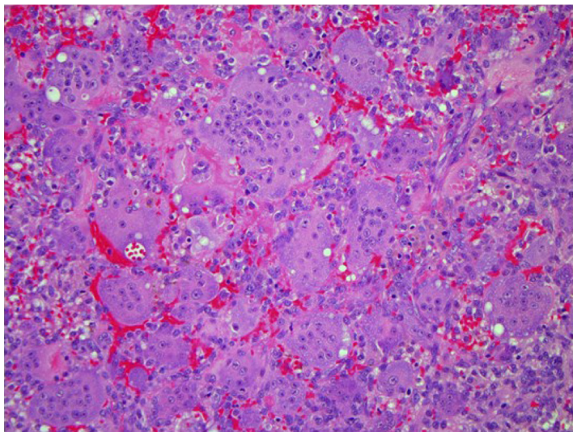

Fig. 4. Untreated giant cell tumour of the bone. There are large numbers of osteoclast-like giant cells evenly distributed throughout the tumour. Interspersed there are mononucleated cells. It is difficult to differentiate the mesenchymal stromal cells from macrophage-like cells; however, the nuclei of the latter approximate those of the osteoclast-like giant cells (haematoxylin and eosin, ×200).

Immunohistochemistry of the diagnostic GCTB biopsy samples demonstrated diffuse RANK staining amongst the osteoclast-like cells with comparatively weaker staining amongst the mononuclear cells (Fig. 6a). The osteoclast-like cells were typically negative for RANKL, with roughly 10–80% of the mononuclear cells expressing this marker (Fig. 6b). Immunohistochemistry of the denosumab treated tumours revealed weak RANK staining amongst the mononuclear cells (Fig. 7a). Staining with RANKL highlighted only rare positivity amongst the mononuclear cells (Fig. 7b). There was no relation between low level residual RANK or RANKL staining after denosumab and local recurrence.

4. Discussion

Since the first report of the efficacy of denosumab for GCTB by Thomas et al. in 2010 [25], several studies about this new targeted therapy for GCTB have been published [23,24,32]. These reports focused predominantly on the safety and efficacy of medical management with denosumab, with limited surgical and histopathological evaluation. A number of diverse radiological tumour response criteria have been used without providing specific details about response or how conflicting outcomes were addressed. Furthermore, no comparative analysis of radiological and pathological findings was performed.

In the present prospective study, we enrolled 20 consecutive patients who presented with resectable but 'high risk' GCTB, based on the likely need for joint resection due to extensive periarticular bone loss, soft tissue mass and/or pathologic fracture. They were treated with preoperative denosumab in order to evaluate its ability to downstage the surgical procedure and allow joint preservation. To be able to draw conclusions about surgical efficacy, clinical, radiological and pathological responses were all analysed. Clinical response to denosumab was overwhelmingly positive with significant pain relief in all 20 patients within the first month of treatment, and complete pain relief in most (16, 80%) by 6 months. This confirms the observations of Martin-Broto et al. that denosumab is effective in decreasing pain in patients with GCTB [32]. There were few and only mild adverse events recorded, consistent with the previously reported safety profile of denosumab [21–23,26,33]. Our comparative radiological observations demonstrated that marked osteosclerosis/bone formation within the lesion,

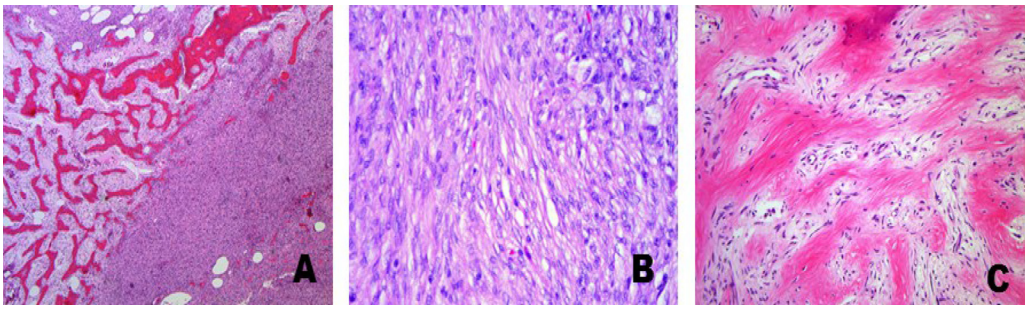

Fig. 5. Giant cell tumour of the bone following denosumab treatment. (A) Low magnification photomicrograph of the residual fibro-osseous lesion highlighting zonation (haematoxylin and eosin, ×50). (B) Higher magnification of the residual fibro-osseous lesion highlighting sheets of bland spindle cells, and (C) areas of spindle cells with interspersed collagen deposition (haematoxylin and eosin, ×200).

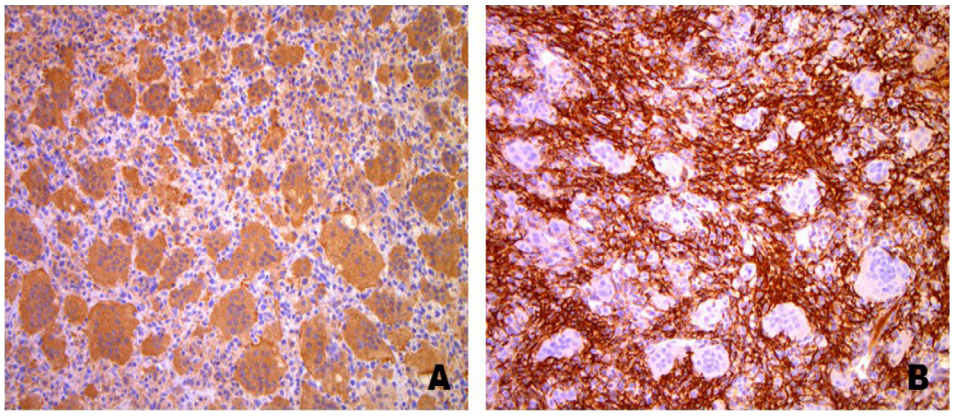

Fig. 6. Untreated giant cell tumour of the bone. (A) There is diffuse and strong expression of RANK amongst the osteoclast-like cells, with comparatively weaker staining in the mononucleated cell fraction, and (B) a RANKL rich region of staining amongst the mononuclear cell population (haematoxylin counterstain, ×200). RANK, receptor activator of nuclear factor kappa B; RANKL, RANK ligand.

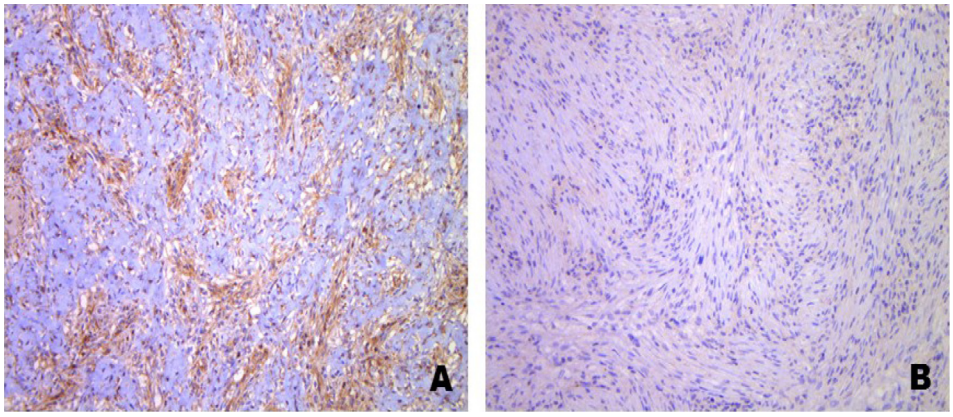

Fig. 7. Giant cell tumour of the bone following denosumab treatment. (A) There is scattered weak immunoreactivity amongst the mononucleated cells for RANK, and (B) the lesion is negative for RANKL (haematoxylin counterstain, ×200). RANK, receptor activator of nuclear factor kappa B; RANKL, RANK ligand.

formation of a sclerotic peripheral rim of bone around the lesion, and thickening of the cortical and subchondral bone as seen on plain radiographs and CT scans reflect the devitalisation of giant cells seen histologically. In addition all pathologic fractures healed following denosumab treatment. The findings at surgery certainly corroborated the denosumab-induced radiological responses. We did not demonstrate a significant effect of denosumab on the size of most lesions, as 11 tumours appeared to enlarge following treatment. This finding is likely multifactorial and related to the increased thickness of the surrounding cortical bone following denosumab, and the fact that there was always some delay between the initial clinical assessment and x rays and the first denosumab injection while waiting for completion of radiological staging studies, biopsy and pathological confirmation of GCTB.

However, as a result of the neoadjuvant treatment with denosumab, a higher rate of joint preserving surgeries (18/20; 90%) were performed than could have been expected based on the initial radiographs, and this was largely related to improvements in cortical and subchondral bone thickness which was evident in 18/20 (90%) patients. In addition overall functional results for patients in this study were very good after preoperative denosumab treatment followed by tumour resection and reconstruction based on all three functional outcome measures which had mean scores above 90%.

The histological results following denosumab treatment in the present study support the results from Branstetter et al. [24], suggesting that denosumab is biologically active in inhibiting osteoclastogenesis, which would be expected of a monoclonal antibody that binds RANKL. Histologic comparison before and after denosumab treatment revealed that the classic architecture of GCTB, consisting of proliferative densely cellular stromal cells as well as RANK-positive mononuclear and osteoclast-like giant cells, had considerably changed. Following denosumab there was an obvious increase in osteoid matrix and woven bone, and the new bone was present with only rare RANK staining amongst the mononuclear cells and focal RANKL positivity. The osteoclast-like cells were rarely detectable.

In a recent *in vitro* investigation of GCTB, it was shown that following denosumab exposure neoplastic mesenchymal stromal cells persist and continue to proliferate although at a slower rate, and lack RANKL expression [34]. Although the stromal cells were quiescent during drug exposure, the neoplastic cells remained proliferative once the microenvironment was free of the RANKL antibody. This suggests that denosumab selectively targets and eliminates the bone destroying osteoclastic cells, but not the neoplastic stromal cells which persist and remain at least partially functional. Another *in vitro* study also found that denosumab lacked antitumour efficacy against giant cell tumour stromal cells, and suggested that tumour recurrence may occur after drug withdrawal [35]. Based on these *in vitro* results, it should not be surprising that our study identified 15% local recurrences (3/20 patients), which occurred at a mean 16 months following surgery. This local recurrence rate did not seem to be affected by neoadjuvant treatment with denosumab, since it was similar to our previous reports of GCTB following surgical management alone [12,13,15]. A recent phase II investigation of the effects of denosumab on GCTB also identified a local recurrence rate of 15% (17/116 patients) following surgical resection [26]. In that study most patients received six additional doses of denosumab following surgery, while no postoperative denosumab was given to any of the patients in our study. This raises the possibility that postoperative denosumab may not be required following preoperative treatment and surgical resection.

Besides this possible biological explanation for local recurrence, the intra-operative findings during intralesional tumour resection are important to consider as well. The typical soft tissue tumour found within the lytic defect of GCTB changed into a gritty fibro-osseous matrix following denosumab which made it difficult at times to determine the true extent of the tumour and the junction between the edge of transformed tumour tissue and normal uninvolved bone. Furthermore, denosumab also lead to thickening of the cortical and subchondral bone surrounding the tumour which provided a strong circumferential bony layer against which curettage and burring could be performed. Although this seemingly positive change facilitated joint preservation surgery by providing support for the joint surface and periarticular geometry, tumour cells may have become entrapped within this thickened new bone. This could have led to tumour being inadvertently left behind following surgical resection, and contributed to the local recurrences. Therefore we would suggest using intra-operative fluoroscopy whenever necessary to facilitate identification of the boundaries of the tumour and denosumab-induced tissue response. In addition, more extensive burring of the surrounding thickened cortical and subchondral bone may be mandatory following initial denosumab treatment. The initial publications from the large prospective phase II denosumab study did not address what happened to participants with GCTB who came off treatment without surgical resection of their tumour [23,25,26,32]. There are few alternatives to denosumab for patients with high-risk or non-operable GCTB. Although two studies have suggested that bisphosphonates may reduce local recurrence following surgical resection of GCTB through its effects on both the stromal tumour cells and osteoclastic cells [9,36], these positive findings were not replicated in a more recent study which used short-term postoperative treatment with bisphosphonates after intra-lesional curettage [37].

A rare but important consideration when treating GCTB is the potential for malignant transformation. This is usually associated with prior irradiation or multiple local recurrences. In our study one patient developed lung metastases diagnosed as benign giant cell tumour, but there were no instances of malignancy. In the initial phase II denosumab publication by Thomas et al., one patient developed a bone sarcoma and another malignant giant cell tumour lung metastases following treatment with denosumab [25]. Aponte-Tinao et al. describe the case of a patient who developed a high grade sarcoma at the site of a previously benign multiply recurrent GCTB during treatment with denosumab [38]. Although it is impossible to determine if these few occurrences of malignant transformation were related to denosumab, this is an important event that needs to be recognised as this treatment becomes used more frequently.

We entered patients into this prospective study because, at the time, there was no effective approved medical treatment available for GCTB, and early evidence from the phase II study suggested that denosumab may significantly benefit patients with this benign but locally aggressive tumour [25]. In our study population, most patients experienced rapid and clinically relevant pain relief. Reported adverse events were uncommon and consistent with the known safety profile of denosumab [23,39]. Together with the previously reported efficacy of denosumab in GCTB [23–26,40], these data suggest that denosumab represents an effective treatment option for patients with GCTB especially when significant surgical morbidity is expected, such as joint resection and endoprosthetic reconstruction [23]. Although this study evaluated only 20 patients, they were enrolled prospectively based on specific entry criteria, and the results clearly demonstrate that all of the patients underwent a less morbid surgical procedure than had been initially planned prior to the positive response to denosumab. The gold standard of treatment for most patients with surgically resectable GCTB remains unchanged – aggressive tumour curettage, high-speed burring and possible use of intralesional adjuvant agents. Denosumab should be considered as an additional and neoadjuvant treatment specifically in difficult and 'high risk' cases to lessen the extent of surgery by facilitating joint preservation, thereby improving functional outcomes and minimising complications. It is important to emphasise that the new osseous tumour matrix and the thickened cortical bone that develop following denosumab treatment raises a new surgical challenge: to delineate the true extent of the tumour. Surgical curettage and burring should be even more thorough following denosumab treatment.

Additional studies are necessary to further evaluate the risk of local tumour recurrence as well as potential long-term effects following denosumab combined with surgical treatment. Cost-effectiveness will also be an important consideration due to the financial impact of including denosumab in treatment protocols for GCTB. Along these lines, it may be reasonable to consider surgery earlier than after the currently suggested six cycles of preoperative treatment, based on serial assessments of cortical and subchondral bone recovery. A shortened treatment approach could still improve rates of joint salvage for high risk patients, but may also have the added benefit of providing less chance for tumour cells to 'hide' within the thickened cortex and subchondral bone which could favourably influence the risk of local tumour recurrence. This approach will require further evaluation through dose-response studies.

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Conflict of interest statement

None.

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