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Clinicopathologic and molecular features of denosumab-treated giant cell tumour of bone (GCTB): Analysis of 21 cases☆

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ABSTRACT

GCTB is an osteolytic, locally-aggressive, rarely-metastasizing tumour, characterized by abundance of osteoclast-like giant cells, induced by neoplastic mononuclear cells expressing high-levels of the receptor activator of nuclear factor Kappa-B ligand (RANKL), a mediator of osteoclast activation. Although the mainstay of treatment is complete tumour removal with preservation of bone, therapy with denosumab, an inhibitor of RANKL, has been introduced for selected cases.

Objectives: Denosumab-treated GCTB (DT-GCTB) was reported to show a wide spectrum of histological changes such as depletion of osteoclast-like giant cells and intralesional bone deposition, which may lead to diagnostic difficulties. We investigated clinicopathologic and molecular features of DT-GCTB, matched with pre-therapy samples.

Participants: 21 cases were included (13 females, 8 males), aged 15 to 64 (median, 30 years).

Results: DT-GCTB showed development of sclerotic neocortex and varying degrees of osteosclerosis radiographically. Marked depletion of giant cells, different degree of ossification, fibrosis, and proliferation of mononuclear cells was observed. Staining for H3.3G34W was positive in mononuclear cells in 19 cases (90.5%), while one negative case was positive for H3.3G34V. H3F3A G34W mutation was confirmed in 17 of 19 cases (89.5%), corresponding to nuclear staining with H3.3 G34W antibody. G34L mutation was detected in one G34W negative case, in which H3.3 G34V nuclear positive staining was observed, possibly due to cross-reaction.

Conclusions: Post-therapy tumours still exhibit a similar mutation profile, while significantly differing from classic GCTB morphologically. Correlation with history of denosumab administration, awareness of features of DT-GCTB, IHC and molecular studies for histone H3 mutations are important in its assessment.

1. Introduction

Giant cell tumour of bone (GCTB) is a rare, locally aggressive, rarely metastasizing neoplasm. It occurs predominantly in the epiphyses of long bones in adults of 20–45 years of age. On imaging, GCTB presents as a lytic, expansile lesion that is frequently eccentric and may be cystic with a well-defined, rarely sclerotic rim. It is characterized by the presence of multinucleated giant cells, or osteoclast-like giant cells (OLGC), which have a characteristic CD33+/CD14- phenotype [1]. The giant cells are attracted from the circulation by the neoplastic

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mononuclear cells expressing high levels of the receptor activator of nuclear factor kappa-B ligand (RANKL), which is a mediator of osteoclast activation [2,3].

At the molecular level, the tumour cells are characterized by a unique interplay between nucleosome H3.3 and telomeres [4]. The mainstay of treatment for GCTB is as nearly complete removal of the tumour as possible with preservation of bone to reduce morbidity. Joint-sparing surgery and careful curettage with the use of adjuvants is the treatment of choice when possible. In situations where the tumour is unresectable or the surgery would lead to excessive morbidity, denosumab adjuvant therapy can be chosen [5].

Denosumab is a human monoclonal antibody to RANKL, which can inhibit the osteoclastic activity of GCTB through OLGC reduction, and in turn has the effect of lack of monocyte recruitment, proliferation, and giant cell formation [6,7]. Denosumab treatment can induce ossification, fibrosis, marked decrease or even disappearance of OLGC [8-10]. The use of denosumab may convert an inoperable tumour to a resectable one by shrinking the extra-osseous component and ossifying tumour and eventually improve functional outcome [11-13].

It has been reported that denosumab treated GCTB (DT-GCTB) shows a wide spectrum of histological changes such as depletion of OLGC and intralesional bone deposition [6,7,11,14]. In this study, we investigated the clinicopathologic and molecular features of DT-GCTB in a monocentric series of 21 cases, which were matched with pre-therapy samples for each case. Those post-therapy tumours differ significantly from the conventional GCTB morphologically, which may lead to difficulty in pathological diagnosis and differential diagnosis.

2. Materials and methods

2.1. Patients

We retrospectively retrieved the clinical records of 21 cases of GCTB at Xijing Hospital from July 2017 to April 2020, and the corresponding pathology samples before and after denosumab treatment. The specimens were fixed in 10% formalin and embedded in paraffin, two of which were decalcified. Haematoxylin-eosin (H&E) stained slides were retrieved. Clinical information including patient's age, gender, tumour location, tumour size, surgical procedure, pathological report, and radiological evaluation was collected from the patients' charts.

2.2. Patient and public involvement

Patients were not involved in the design of this study. Fully anonymized pathology material and reports as well as radiology material and reports were used. This was a retrospective one-time clinical event study, so no informed consent was obtained, according to the author's institutes ethical guidelines. The results will be disseminated by sharing the open access publication upon publishing.

2.3. Histologic evaluation

H&E slides were independently evaluated by two pathologists (H. C. and L. Y.). In case of disagreement, a consensus was reached by a revisit of the slides, using a double-headed microscope, or telepathology with a third pathologist (P.C.W.H.). Giant cells and mitosis were counted on ten consecutive high-power fields (field surface: 0.1734mm^2; lens magnification: ×400) in areas with the highest density of DT-GCTB.

2.4. Immunohistochemistry (IHC)

In all cases, a representative block of the formalin-fixed paraffin-embedded tissue was selected. Sections of 4-μm thickness were cut and then immersed in a 10-mM sodium citrate buffer (pH 6) for 20 min at 97 °C for dewaxing and antigen retrieval. The following primary antibodies were used: H3.3 G34W (rabbit monoclonal, clone RM263; RevMab BioSciences, San Francisco, CA, USA), H3.3 G34V (rabbit monoclonal, clone RM307; RevMab BioSciences, San Francisco, CA, USA), and H3.3 G34R (rabbit monoclonal, clone SD347; RevMab BioSciences, San Francisco, CA, USA). The staining was performed using an automated Roche Auto Stainer (Roche). Only nuclear staining was scored for all antibodies. Appropriate positive and negative controls were used.

2.5. Mutational analyses of H3F3A

DNA was obtained with a KingFisher mL extractor (Thermolab System) according to the manufacturer's instructions. Exon 1 of H3F3A was amplified by polymerase chain reaction with the following primer sets: H3F3A forward, 5′-TAAAGCACCCAGGAAGCAAC-3′; H3F3A reverse, 5′-CAAGAGAGACTTTGTCCCATTTTT-3′. Polymerase chain reaction conditions were as follows: 95 °C for 7 min; 40 cycles at 95 °C for 30 s, 60 °C for 45 s, and an extension step of 45 s at 72 °C; and the last cycle at 72 °C for 10 min. After purification, the DNA was labelled with the Big Dye Terminator v1.1, and the sequencing was performed on a 3130 Genetics Analyzer.

3. Results

3.1. Clinical findings

Patients' age at the time of surgery after denosumab treatment ranged from 15 to 64 years. The mean age was 33 years, and the median age was 30 years. The male to female ratio was 0.62:1. The sacrum was the most involved bone, probably due to selection by the treatment. Tumour specimens obtained before treatment included biopsies (n = 9) and curettages (n = 12). Initial surgical treatment was not feasible for two patients. For the other patients, denosumab was indicated for tumour reduction to decrease post-surgery morbidity. After denosumab treatment, complete surgical excision (n = 11) and curettage (n = 10) specimens were obtained. Tumour sizes ranged from 4.3 to 13 cm. The mean tumour size was 6.2 cm, and the median was 5.6 cm. At the onset of treatment, 4 patients presented with relapsing tumours. Follow-up duration ranged from 5 to 37 months with a median of 17 months. None of the patients experienced side effects or complications related to denosumab therapy. There was no malignant transformation in our case series. Twenty patients had complete remission and one patient (case 8) developed recurrence four months after the tumour curettage and post-surgery denosumab adjuvant therapy. Tumour location, surgical procedures, tumour status (primary or relapse) and follow-up data are listed in Table 1.

The tumour response to the treatment was evaluated radiographically. Pre-treatment tumours were epiphyseal, expansile, osteolytic and ill-defined, and lacked well-demarcated borders (Fig. 1A). In relapsed GCTB after curettage and bone grafting, the tumours were larger than before (Fig. 1B). The DT-GCTB showed the development of the sclerotic neocortex and varying degrees of osteosclerosis. The tumours were well-demarcated and exhibited ossification and sclerotic rim (Fig. 1C).

3.2. Histological findings

The histology was assessed for the presence of residual GCTB, tumour appearance and proportion of mononuclear stromal cell component and bone component. Histological features including OLGC, cytologic atypia, reactive ossifying rim, necrosis, infarction, foamy macrophages, inflammatory cell infiltrate, hyalinization, sclerosis, hemosiderin pigment, cystic change, hemangiopericytoma (HPC)-like vessels, oedematous areas and others were evaluated (Table 2 and Supplemental Table 1).

There were two main components in the DT-GCTB, mononuclear stromal/spindle cell component and ossification component. Within the 21 cases, the ossification component predominated in 9 cases (42.9%), while the mononuclear stromal/spindle cell component predominated

Table 1
Clinical data of the GCTB cases ($n = 21$).

Variables	Results
Age (median; range) (years)	30; 15–64
Gender, n (%)	
Female	13/21 (61.9)
Male	8/21 (38.1)
Tumour size (median; range) (cm)	5.6; 4.3–13
Localization, n (%)	
Sacrum	6/21 (28.6)
Lumbar vertebrae	3/21 (14.3)
Thoracic vertebrae	3/21 (14.3)
Ilium	2/21 (9.5)
Radius	2/21 (9.5)
Tibia	2/21 (9.5)
Ischium	1/21 (4.8)
Femur	1/21 (4.8)
Pelvis	1/21 (4.8)
Recurrent tumour before denosumab, n (%)	4/21 (19)
Predenosumab samples, n (%)	
Biopsy	9/21 (42.9)
Curettage	12/21 (57.1)
Postdenosumab samples, n (%)	
Curettage	10/21 (47.6)
Surgical excision	11/21 (52.4)
Denosumab indications, n (%)	
Surgery not possible initially	2/21 (9.5)
Tumour reduction	19/21 (90.5)
Neoadjuvant denosumab duration (median; range) (months)	1; 1–25
Postdenosumab surgery, n (%)	21/21 (100)
Adjuvant denosumab, n (%)	10/21 (47.6)
Adjuvant denosumab duration (median; range) (months)	18.5; 4–24
Post-denosumab follow up, n (%)	
Disease free	20/21 (95.2)
Progression/relapse	1/21 (4.8)

in 7 cases (33.3%), and the two components were equal in quantity in the other 5 cases (23.8%). There was complete elimination of OLGC in 7 of the cases (33.3%) (Fig. 2A). Focal residual classic GCTB was present in 3 cases (14.3%); all cases showed depletion of OLGC, except in the foci of residual classic GCTB (Fig. 2B). In addition, in the 21 cases, reactive woven bone at the periphery of the tumour was observed in 9 cases (42.9%); the reactive bone was osteoid deposit merged with the native cortical bone. Local tumour invasion into the surrounding soft tissue was observed in 4 cases (19%), one of which demonstrated focal vascular invasion (Fig. 2C). The other features observed in DT-GCTB included: tumour necrosis in mononuclear cells (6/21, 28.6%), infarction (5/21, 23.8%), haemorrhage along with oedematous changes (7/21, 33.3%) and inflammatory cell infiltrates (18/21, 85.7%) (Fig. 2D).

The DT-GCTB cases were observed to have many morphological variations that could mimic other lesions. The spindle cells could be arranged in a storiform pattern (5/21, 23.8%), along with a collection of foamy macrophages in some of the cases (12/21, 57.1%), mimicking fibrous histiocytoma (Fig. 2E). HPC-like vessels were observed in 11 cases (52.4%), mimicking solitary fibrous tumour, previously called HPC (Fig. 2F). Few cases showed epithelioid cells arranged in the sheet-like deposition of matrix resembling collagenous or osteoid stroma (3/21, 14.3%), mimicking sclerosing epithelioid fibrosarcoma (Fig. 2G). Besides, mild nuclear atypia including nuclear enlargement, hyperchromasia and moderate pleomorphism was noted in three cases (14.3%) (Fig. 2H). Cystic change in stroma was observed in 11 cases (52.4%) (Fig. 2I), one of which had cystic spaces lined by OLGC exhibiting prominent aneurysmal bone cyst (ABC)-like changes. Storiform bundles of collagen were observed in five cases (23.8%) (Fig. 2J). The clinical, morphological, immunohistochemical features and molecular studies of DT-GCTB cases were summarized in Table 2.

Fig. 1. Representative radiological findings (case 19). A. Pre-treatment lesion in right distal radius proven to be GCTB. B. Three months after curettage and bone grafting, the lesion recurred, with larger size than before (short arrow, bone grafting area; long arrow, osteolytic area). C. After treatment with three cycles of denosumab, the tumour was well-demarcated and exhibited ossification and sclerotic rim.

Table 2
Histological, immunohistochemical features and molecular studies of DT-GCTB cases.

Variables	Results, n (%)
Residual OLGC	
>1%	2/21 (9.5)
≤1%	1/21 (4.8)
Occasional OLGC	11/21 (52.4)
Complete absence	7/21 (33.3)
Necrosis	
No necrosis	10/21 (47.6)
Tumour necrosis in mononuclear stromal cells	6/21 (28.6)
Infarction	5/21 (23.8)
Predominant component	
Ossification	9/21 (42.9)
Mononuclear stromal cell	7/21 (33.3)
Equal	5/21 (23.8)
Storiform pattern of spindle cells	5/21(23.8)
Residual GCTB	3/21 (14.3)
Epithelioid mononuclear stromal cells in sheets	3/21 (14.3)
Cytologic atypia	3/21 (14.3)
Presence of bone rim	9/21(42.9)
Foamy macrophages	12/21 (57.1)
Inflammatory cell infiltrate	18/21 (85.7)
Hyalinization/ storiform collagen	5/21 (23.8)
Hemosiderin	10/21 (47.6)
Cystic change in stroma	11/21 (52.4)
HPC-like vessels	11/21 (52.4)
Oedematous areas/mucoid degeneration	7/21 (33.3)
ABC-like changes(cystic spaces lined by OLGC)	1/21 (4.8)
Infiltrate surrounding soft tissue	4/21 (19)
Giant cells/10HPF (median; range)	2; 0–95
Mitotic index/10HPF (median; range)	1;0–5
H3.3 G34W IHC	19/21(90.5)
H3.3 G34V IHC	1/21 (4.8)
H3.3 G34R IHC	0/21 (0)
H3F3A mutation	18/19(94.7)

3.3. Immunohistochemical stain

IHC staining for H3.3G34W was performed in all cases after denosumab treatment. H3.3 G34W stain showed diffuse nuclear staining pattern in mononuclear cells, but not in OLGC (Fig. 2K). The staining was positive in 19 cases of 21 cases (90.5%). The two H3.3G34W negative cases were stained with H3.3G34V and H3.3G34R antibodies, one of which showed positive nuclear staining for H3.3G34V (Fig. 2L).

IHC staining was performed only in 15 pre-treatment cases, which was positive in 14 cases (93.3%). Denosumab did not significantly alter the H3.3G34W IHC staining pattern. Therefore, abundant H3.3G34W positive mononuclear cells remain in post-denosumab-therapy tumours can be considered as one of the important features of DT-GCTB.

3.4. Mutation of H3F3A

The *H3F3A* mutation status was verified by direct Sanger sequencing in 19 post-denosumab-therapy cases except for two decalcified specimens due to DNA degradation. G34W mutation was confirmed in 17 of the 19 cases (89.5%), corresponding to the nuclear staining with H3.3 G34W antibody in these cases. G34L mutation was detected in one of the G34W negative cases (case 16), in which H3.3 G34V nuclear positive staining was observed (Fig. 2L), possibly due to cross-reaction. Another G34W negative case (case 17) turned out to be H3.3 wild type, which was re-reviewed and the diagnosis of GCTB confirmed by PCWH. H3F3A mutation status, together with clinical, morphological, IHC findings of DT-GCTB are outlined in Table 2.

4. Discussion

GCTB may show a spectrum of morphological changes after denosumab treatment. Features of DT-GCTB include OLGC depletion, massive intralesional bone deposition and collagenous matrix, and proliferation of bland spindle cells replacing mononuclear tumour cells, which bear little morphologic resemblance to GCTB [6,7,9,11,14,15]. Without knowledge of prior treatment of denosumab, DT-GCTB can be misdiagnosed as a range of different tumours, from benign fibro-osseous lesions to osteosarcoma [7,16].

In our study, 33.3% of the cases showed complete elimination of OLGC, an important feature of DT-GCTB reported in previous studies. The absence of OLGC makes the diagnosis challenging for inexperienced pathologists, particularly when the denosumab treatment history is missing. Residual classic GCTB (comprising>1% of residual tumour) at the tumour's periphery was observed in 14.3% of the cases, with OLGC smaller in size and containing fewer (up to 10) nuclei. Only a few studies have reported a substantial amount of residual GCTB in a small subset of their cohorts [17,18]. The amount of residual OLGC did not correlate with the duration and dose of denosumab. Tumours in our cases demonstrated varying proportions of residual OLGC although the treatment course was similar. These findings correlate with a recent study of the French bone pathology group [14]. The suggested correlation with the number of giant cells and response in their series could not be substantiated in our series.

We also observed cytologic atypia in a small proportion of mononuclear cells in 3 of our cases (14.3%), like other reported series, sometimes reported as symplastic changes in GCTB [14,19], but none of which underwent malignant transformation. These atypical cells, only seen in post-treatment cases showed irregular nuclei with enlargement, moderate pleomorphism and rare mitoses, which can be easily mistaken for osteosarcoma especially in the presence of osteoid deposition. Wojcik et al. found cytologic atypia to be a feature seen in early denosumab treatment [7]; however, we were not able to confirm this finding in our series, in the patients with similar denosumab treatment duration. Recently several studies point to the marked histological changes following denosumab administration and marked bone formation [16,18,20-22]. Interestingly the expression of H3.3G34W post treatment appeared to be decreased pointing to a diminished antigenicity of the mononuclear neoplastic cells [22], while the expression of SATB2 appears unchanged.

The mononuclear cells in most of our cases were spindle-shaped except in 3 cases that exhibited epithelioid morphology. In one of these 3 cases, sheets of epithelioid cells within collagenous stroma resembled somewhat sclerosing epithelioid fibrosarcoma. The collection of foamy macrophages was a frequent finding and its combination with storiform pattern of mononuclear cells is like that seen in non-ossifying fibroma. In some cases, extensive hyalinization and sclerosis in the stroma may mimic the desmoplastic stroma of bone. It has been reported that abundant dilated vascular channels was observed in a few cases, which can raise a concern for intraosseous haemangioma [18]. We also observed HPC-like vasculature both in spindle cells area and hypocellular stroma, which may impart the appearance of solitary fibrous tumour, although the latter usually does not primarily involve bone. In cases with cystic spaces lined by OLGC, ABC-like changes might already be present in pre-treatment tumours, which was previously described as secondary ABC.

The variable morphological changes induced by denosumab treatment can cause diagnostic difficulties in pathology. The pseudosarcomatous changes, including high cellularity, cellular atypia, and new bone deposition with OLGC depletion with little reminiscence to the classical GCTB, may lead to an erroneous diagnosis of osteosarcoma [7,16,23]. As a matter of fact, case 1 in our series was initially misdiagnosed as osteosarcoma by a less experienced pathologist before the multidisciplinary discussion. Under this circumstance, it is essential that the pathologist is informed of a treatment of denosumab to avoid misdiagnosis.

Hayashida and coworkers clearly showed that following stopping of Denosumab treatment the morphologic appearance was reversed

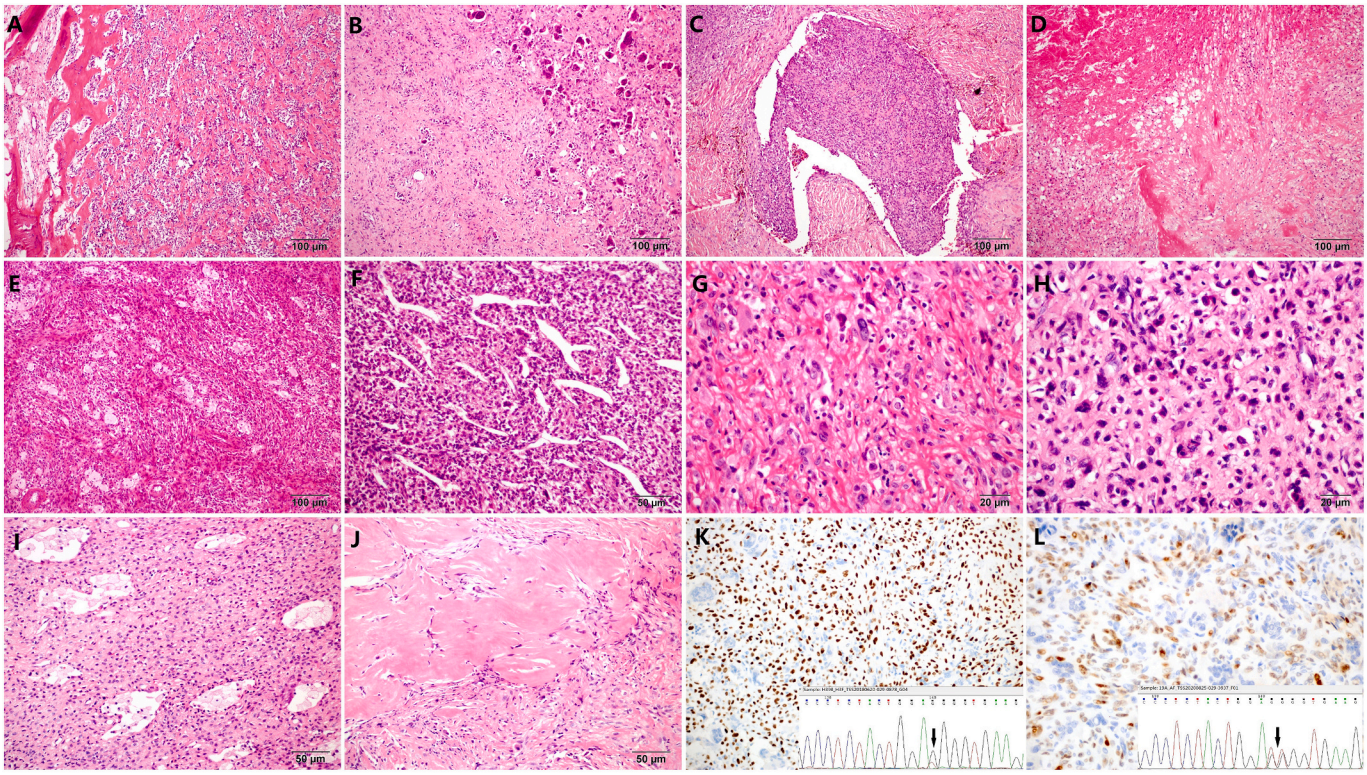

Fig. 2. Histological features and immunohistochemical stains of the DT-GCTB. A. Reactive bone at the periphery of the tumour, with complete absence of OLGC. B. Focal residual classic GCTB along with area of bone formation and OLGC depletion in DT-GCTB. C. Focal vascular invasion in one case. D. Necrosis and haemorrhage along with oedematous changes and inflammatory cell infiltrate. E. Spindle cells arranged in storiform pattern along with collection of foamy macrophages, mimicking fibrous histiocytoma. F. HPC-like vessels in focal areas. G. Epithelioid cells arranged in sheet-like deposition of matrix resembling collagenous or osteoid stroma, mimicking sclerosing epithelioid fibrosarcoma. H. Mild nuclear atypia in mononuclear stromal cells. I. Cystic change in stroma. J. Storiform collagen. K. IHC of H3.3 G34W stain in mononuclear cells. G34W mutation was detected in *H3F3A* (insert: c.103G > T mutation, Sanger sequencing). L. IHC of H3.3 G34V in mononuclear cells in case 16. G34L mutation was detected in *H3F3A* (insert: c.103GG > TT mutation, Sanger sequencing).

implying the transient treatment effect on the bystander cells and not on the primary tumour cells [21].

On the cytogenetic level, GCTB is characterized by the presence of telomeric association by a yet unknown mechanism [24,25]. At the molecular level, a driver mutation in histone gene *H3F3A*, most commonly p.G34W, has recently discovered in GCTB [26-28]. Immunohistochemical staining with the mutation-specific antibody H3.3 G34W has proven to be a useful tool for the diagnosis of GCTB with high sensitivity and specificity [28-31]. The H3.3 G34W is positive in 85–92% of GCTB cases [30,32,33]. A small number of GCTB cases carry other H3.3 mutations [31]. In our study, the H3.3 G34W antibody stained 90.5% of the cases. Two cases were negative for H3.3 G34W, one of which had H3.3 G34L mutation although IHC for H3.3 G34V was positive due to cross reaction [34], while the other case had wildtype *H3F3A*. A possible cross reaction between H3.3 G34V antibody and G34L mutant protein is an interesting phenomenon, and may become a potential diagnostic pitfall. Similarly, a recent report has pointed out that H3G34-mutant specific antibody is not a perfect surrogate for the H3.3 mutation in brain tumours [35].

P63, SM-ACT, RANKL and NFATc1, valuable markers in GCTB diagnosis have limited usage in DT-GCTB cases due to loss of their expression after treatment [8,36]. H3.3 G34W IHC stain and molecular tests for *H3F3A* mutations are in contrast very helpful in DT-GCTB assessment [9]. Most DT-GCTB cases express strong H3.3 G34W in the residual mononuclear tumour cells. In our series, molecular studies confirmed the persistence of the *H3F3A* G34W mutation after denosumab treatment (18/19, 94.7%) and were consistent with the IHC staining results with the anti-H3.3 G34W antibody. In addition, the H3.3 G34W IHC stain can highlight the neoplastic cells rather than reactive components in DT-GCTB [19], in which the true boundaries of the tumour are difficult to delineate due to its metamorphic transition into the adjacent reactive woven bone and connective tissue [11].

Long-term use of denosumab can cause complications including new malignancy, tumour progression, osteonecrosis of the jaw, and others [37,38]. Malignant transformation after the use of denosumab has been reported in a few studies, in which G34W mutations are present in all pre-denosumab, post-denosumab, and malignant samples and IHC stain and *H3F3A* mutation study have no value in identifying malignant transformation in DT-GCTB [14,39,40]. We did not find any malignant transformation in our case series, and no complication or side effect of denosumab treatment was observed in the case series within the time frame studied. The most common location of the tumours in this series was the sacrum. This differs from the distribution of GCTB in general and is due to selection of cases for denosumab treatment. This treatment option is offered in cases of difficult surgery because of tumour extent or location like the sacrum.

In summary, DT-GCTB exhibits a wide spectrum of morphology, including a striking giant cell depletion and massive intralesional bone deposition with a rim of reactive bone, without a noticeable effect on the contingent of neoplastic mononuclear cells. While the post-therapy tumours can differ significantly from the classic GCTB morphologically, they still exhibit a similar histone H3 mutation profile. Multidisciplinary correlation along with the clinical history of denosumab administration, awareness of the histological features of DT-GCTB, and IHC and molecular studies for histone H3 mutations are important in the assessment of DT-GCTB.

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Data sharing statement

No additional data available.

Ethical approval

Because fully anonymized pathology material and reports as well as radiology material and reports were used, and as it is a retrospective of a one-time clinical event, no informed consent was obtained. This is according to the author's institutes ethical guidelines.

Credit authorship contribution statement

LY collected the material of the cases and wrote the first draft of the manuscript; HZ: was responsible for mutation detection; XZ revised the manuscript; YT provided the image information; ZW and HH provided the clinical information; YW performed all immunohistochemical staining; XF and JL participated in collecting the material of the cases; PH reviewed and revised the manuscript and was involved reviewing cases especially in the G34W negative case (case17) to confirm the diagnosis; HC was involved in the study design, data collection and analysis, pathology diagnosis and revising the manuscript. All authors have read and approved the final manuscript.

Declaration of competing interest

All authors declare that there is no conflict of interest.

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