Giant cell tumor in a case of Paget's disease of bone: an aggressive benign tumor exhibiting a quick response to an innovative therapeutic agent

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Summary

Giant cell tumor of bone, also called osteoclastoma, is a rare skeletal complication of Paget's disease of bone. We here report a patient from Southern Italy who developed a GCT infiltrating the neighboring tissues. We will focus on either a review on this rare bone tumor, including some genetic aspects, or the current established therapies. Since this case has been published in International literature, here we report the updated clinical findings on it. Finally, we will describe the therapeutic outcomes of this unique complication of Paget's disease of bone as a rapid response to an innovative therapeutic agent.

KEY WORDS: Paget's disease of bone; giant cell tumors; osteoclastoma; RANKL/RANK/OPG; anti-RANKL antibody.

Introduction

Paget's disease of bone (PDB) is an alteration of the focal bone remodeling in which the normal skeletal architecture is replaced by a not organized bone tissue, with a tendency to deformities and fractures. Although reported, the occurrence of malignancies, including osteosarcoma, chondrosarcoma and fibrosarcoma, as a complication of PDB, is an uncommon event (<1%).
The giant cell tumor (GCT) is a rare complication of PDB (1), usually associated with long standing polyostotic disease (2-5). Compared to the traditional, nonpagetic, form, PDB-GCT reaches a peak of incidence at older ages (third vs. sixth decade), with a slight predilection for males and more frequently localizes in the craniofacial bones, less often pelvic and vertebral. Its location at the ends, typical of traditional GCT, is unusual (5-10) (Table 1).
Solitary or multiple forms have been described, sometimes exhibiting a peculiar geographical distribution and/or a familial pattern. In particular, some studies report a significant increase in the prevalence of PDB-GCT in Campania, specifically nearby Avellino (6, 11, 12); the patient, object of this study, comes from Naples, the capital town of Campania.
Although GCT is usually a histologically benign tumor confined to the bone, along with indolent behavior, it may sometimes show an infiltrative pattern of growth with an involvement of soft and/or visceral tissues (3, 13-17). However, an its malignant degeneration appears to be a rare event (4).

Hypotheses for the etiopathogenesis of PDB

Currently, the primary cause of PDB is still unknown and viral and genetic hypotheses need of clear demonstration.

Viral hypothesis

A viral etiology has been proposed for many years, based on the discovery of virus-like intranuclear inclusion bodies in osteoclasts (OCLs) of pagetic bone (18-21). Myrrh and Gold also reported a case where virus-like intranuclear inclusion bodies were found in the OCL of PDB-GCT (22). Unfortunately, many other reports have not replicated similar findings in their analyzed series (23-26).

Genetic hypothesis: all the gene products involved in the pathogenesis of PDB and PDB-like syndromes are important regulators/modulators of osteoclastogenesis and/or metabolic osteoclast activity.

Recently, germline mutations in the gene encoding p62 protein (*SQSTM1/p62* gene) have been identified in patients with sporadic and familial PDB (15, 27-30). In general, it has been demonstrated that 12-40% of PDB index cases have at least 1 first degree relative affected by PDB (31), who exhibit a 7–10 times increased risk to develop PDB with respect to general population. This risk is even greater in relatives of patients with deforming disease and those with an early age at diagnosis (32).
The protein p62 is involved in the signal cascade that involves the RANK-dependent signaling, essential for osteoclastogenesis (33).

Table 1 - Main features of PDB-GCT vs. traditional GCT.

	PDB-GCT	Traditional GCT
AGE	60	20-40
SEX PREVALENCE	M	F
LOCALIZATIONS	Skull – Facial bones Pelvis – Spine Rare in long bones (metaphysis diametaphysis diaphysis)	Long bones in epiphysis/ metaphysis: Distal Femur Proximal Tibia Distal Radius

Table 2 - Mutations and polymorphisms in other genes than *SQSTM1/p62* gene identified in patients with PDB and correlated syndromes.

Gene	Type of mutation/polymorphism	Encoded product	Disease
TNFRSF11A	-Activating germline mutations	Receptor Activator of Nuclear factor KB (RANK)	Familial Expansile Osteolysis (FEO); Early-onset PDB (EO-PDB); Expansile Skeletal Hyperphosphatasia (ESH)
TNFRSF11B	-Homozygous inactivating mutations -Single Nucleotide Polymorphism (SNP)	Osteoprotegerin (OPG)	Juvenile PDB; Predisposition to PDB in subjects without germline mutation of SQSTM1/p62
VCP	-Inactivating mutations	Valosin-containing protein	Inclusion body myopathy, classic PDB and fronto-temporal dementia (IBMPFD)

In particular, protein p62 is involved in signal transduction along the NFkB pathway and an abnormal functioning of this protein may result in abnormal activation of NFkB and hence in increased of both osteoclastogenesis and metabolic activity of OCL (34).
In addition to *SQSTM1/p62* gene, mutations and polymorphisms in several other genes, encoding components of the "RANKL-RANK-NFkB pathway, have been identified in patients with PDB and correlated syndromes (35-40) described in Table 2.
However, how PDB-GCT may develop is not clear yet. It is reasonable to assume that PDB-GCT may develop as a result of abnormal and excessive localized osteoclastogenesis, associated with stromal cell proliferation with possible additional molecular alterations, not defined (2).

Hypothesis for the etiopathogenesis of GCT: RANKL-RANK-NFκB pathway and the development of GCT

After the identification of the cytokine Receptor Activator of Nuclear factor κB ligand (RANKL), an important osteoclastic differentiating factor, great advances have been achieved in understanding the pathogenesis of GCT, in general (15).
Many studies would identify RANKL as highly expressed by stromal cells within the GCT tissue (41, 43, 38-40). stromal cell would be the "neoplastic driver" and RANKL would appear to be essential in the pathogenesis of GCT (15). The genetic basis underlying RANKL overexpression by stromal cells have not been identified, and abnormalities of the *RANKL* gene have not been found in GCT specimen (15).
It is possible that reciprocal, unidentified, signals from giant cells may be involved in maintaining an immature state of the stromal cell and would be required for the expression of RANKL (15).
GCT is clinically characterized by osteolytic lesions able to spread out, and histologically by the presence of multi-nucleated giant cells similar to OCLs. Several authors believe that the mononuclear stromal cells represent the neoplastic component of GCT able to produce molecular signals which promote the formation of multinucleated osteoclast-like cells. The benign multinucleated giant cells, stimulated by the neoplastic mesenchymal component of GCT, promote the process of osteolysis (7, 44, 45). The tumor cells express RANKL and its receptor RANK. Thus, the pathway RANKL/RANK is an essential mediator for the activity, the formation and survival of OCLs (46-52).

GCT: Instrumental diagnosis, current therapeutical approaches and future perspectives

The instrumental diagnosis of GCT mainly relies on radiological surveys such as conventional X-rays (2), CT (2, 53) and MR (2, 54-56).

Table 3 - Denosumab: an innovative drug for the management of patients with post-menopausal osteoporosis and the following conditions with bone loss or destruction.

Conditions featured by bone loss/destruction treatable with Denosumab
Drug use inducing bone loss (hormone ablation therapy, exposure to chronic glucocorticoids and immunosuppressants therapies)
Rheumatoid arthritis
Osteolytic bone metastases

The therapeutic management of GCT is not well codified and may be represented by surgical removal of the mass (15, 57-64) and/or radiotherapy (15, 19, 45, 65-68), and/or selective arterial embolization (69-71) and/or pharmacotherapy (Interferon-α) (65, 72, 73) and/or amino-bisphosphonates (15, 74-79) and/or steroids (9-11, 18, 79, 80).
However, as above reported, the increased knowledge on the RANKL-RANK pathway has allowed the development of new therapeutic modalities such as the one represented by human monoclonal antibody anti-RANKL agent: denosumab (47).

Denosumab

It is a fully human monoclonal antibody, IgG2, specific against RANKL, able to: 1) prevent its binding to RANK; 2) inhibit the development of OCLs and their activity; 3) reduce bone resorption; and 4) increase the bone density (81-86). Denosumab is therefore an innovative therapeutic agent for the management of patients with post-menopausal osteoporosis (86) and conditions with bone loss or destruction (86-92), as reported at Table 3.
Based on these considerations, it is expected that denosumab will represent a well-tolerated therapy for patients with GCT, relapsed or not surgically treatable, or for patients with surgically treatable disease whose surgery, originally scheduled during the study, is associated with severe morbidity.
This evaluation is still ongoing in a multicentre, international, open phase II trial on patients with bone GCT receiving 120 mg of denosumab, sub-cutaneously (SC) administered, every 4 weeks (Q4W) with a loading dose of 120 mg, SC at day 8 and 15 of the study, in combination with daily 500 mg of calcium and 400 IU of vitamin D (EudraCT Code: 2008-001606-16).
Previously, Thomas et al. (85, 86), in 37 patients with surgically untreatable or recurrent GCT, showed a good tumor response and a good tolerability to denosumab in 86% of cases, as reported at Table 4. In 33 patients (89%) minor adverse events occurred (the

Table 4 - Results of treatment with denosumab on 37 patients with surgically untreatable or recurrent GCT.

1)	Clinically: pain reduction, improvement of functional status with increased motility and return to work;
2)	Histologically: regression or complete elimination of giant cells associated with reduction in RANKL expression within the tumor;
3)	Radiographically: reduction-stabilization of the tumor inside the bone, reducing the mass of tissue growing outside the bone and formation of new bone;
4)	Biochemically: suppression of bone turnover markers: urinary N-telopeptide and serum C-telopeptide

Table 5 - Clinical features of the male patient exhibiting PDB-GCT. The age of the diagnosis of PDB was 38 years whereas the GCT developed at 68 years. Her daughter was also affected by polyostotic PDB at 20 years.

Diseases
Polyostotic PDB
Obesity
Diverticulosis
Benign prostate hyperplasia
Mild hearing loss
Hematuria
PDB-GCT

most commonly represented by headache and nasopharyngitis), no serious adverse events related to the form of treatment or death were reported during this study. No patients developed antibodies anti-denosumab (85, 86).
Therefore, further studies on denosumab, as a new therapeutic agent for GCT, are needed (85, 86).

Case Report

The clinical description of this PDB-GCT case has been recently published (93). Tables 5-8 summarize the main clinical and therapeutical features reported (93). Figures 1-3, unpublished, described the findings of technetium-99m-labeled bisphosphonate bone scintigraphy, abdomen-pelvic CT and 3D-CT scan of the tu-

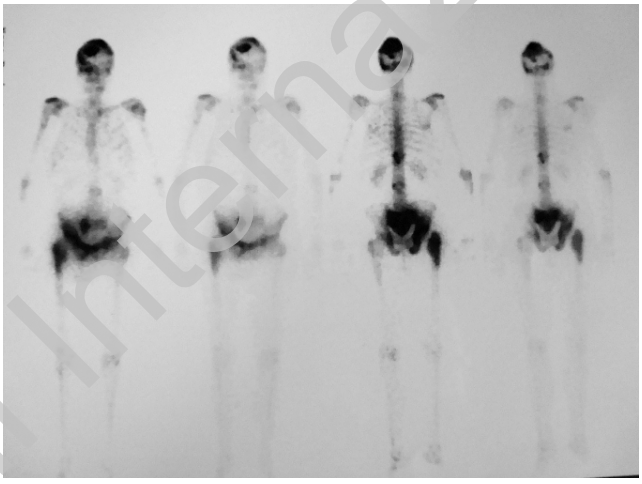

Figure 1 - Initial total body bone scintigraphy, performed before any therapy, evidenced several hyperactive areas: skull, vertebral bodies, pelvis and both femurs. All these data were suggesting a polyostotic pagetic involvement.

Table 6 - Physical, radiological and bone turnover examinations in the PDB-GCT case (93).

Physical examination	Abdominal-pelvic US and CT	Pelvis and lower limbs X-rays	Total Body bone scintigraphy	Abnormal bone turnover findings
-Initial mild and localized back pain (worsened by physical activity), progressively worsened and extended to the lumbar and left iliac region -Firm mass extending from the left iliac bone to the anterior abdominal region	-Lesion (diameter of approximately 8 cm) with irregular margins, and tightly adherent to the ileum and left ischio-pubic bones -The lesion infiltrated the rectum, the distal portion of the left ureter and the bladder	-Cortical thickening and sclerosis of the multiloculated lytic lesions of the iliac bones -The cortex of the long bones of both upper and lower extremities was coarse and thickened	Increased uptake in skull, vertebral bodies, proximal regions of the humerus, pelvic bones, and proximal femurs bilaterally	-Very high activity of total serum alkaline phosphatase (1.346 IU/L; normal range, 8–300 IU/L) -Very high activity of bone-specific fraction (230.3 IU/L; normal range: 6-16 IU/L)

Table 7 - Findings at biopsy of pelvis and bladder (cystoscopy for hematuria) reported in the PDB-GCT case (93).

- Presence of giant multinucleated cells consistent with GCT diagnosis;
- The cell population showed a granulomatosis-like pattern of appearance, associated with lympho-monocyte and eosinophilic components;

} compatible with ab extrinseco infiltration by the GCT

- Complete integrity of urothelial epithelium, whereas the chorion and muscolar tunica showed an infiltrate constituted by monuclear cells and giant multinucleated cells within an edematous-myxoid stroma.

Table 8 - Treatments initially performed on PDB-GCT subject (93): positive and negative results.

Treatments (in chronological order)	Dosage	Extension of treatments	Positive results	Negative results
Intramuscular injections of dexamethasone + Intramuscular clodronate	8–12 mg/day 100 mg	3 weeks Every 3 days for the first 3 weeks, then weekly	Good clinical response: -normalization of serum ALP; -pain resolution after 1 week. Patient remained pain-free: -After 1 month of therapy: reduction in size of the pelvic mass (pelvic CT) -After 6 months: substantial stabilization of the disease (CT) -Gradual reduction, until normalization, of serum ALP (216 UI/L; normal range, 8–300 IU/L)	-Persistence of the parietal bladder infiltration (pelvic CT). -Occurrence of corticosteroid side effects -High vascularization of the tumor on angiography -After suspension of corticosteroid therapy, the patient restarted experiencing pain in sacral region, associated with left leg claudicatio -New rise in serum ALP levels (695 UI/L; normal range, 8–300 IU/L)
Oral dexamethasone	0.75 mg/day (daily maintenance dose)	Subsequent 5 months		
Selective arterial embolization	N. A.			Three months after the embolization, the lesion remained stable
Intravenous infusion of zoledronic acid	4 mg	Every 3 weeks for 4 cycles	-Good control of bone pain -Normalization of the serum ALP	-No effect on the tumor size.

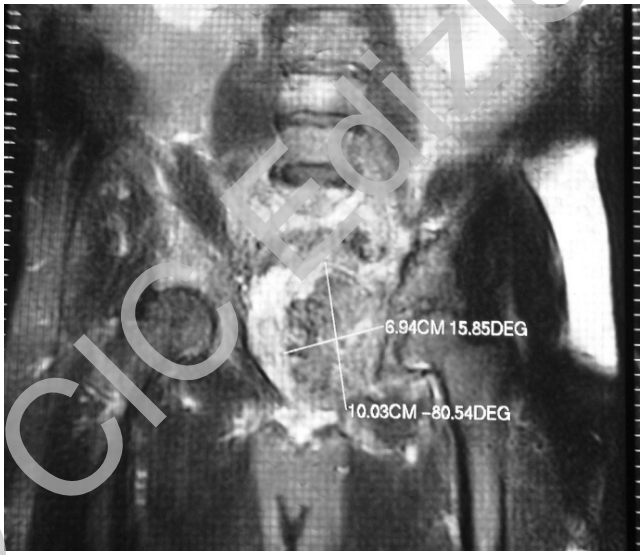

Figure 2 - First pelvic MR shows an extended osteo-destroying lesion of left ileal and ischio-pubic branch associated with a huge solid neoformation mostly occupying the pelvis and compressing local muscles, prostate and bladder. The diffuse abnormal signal of the skeletal pelvic segments was agree with a pagetoid aspect.

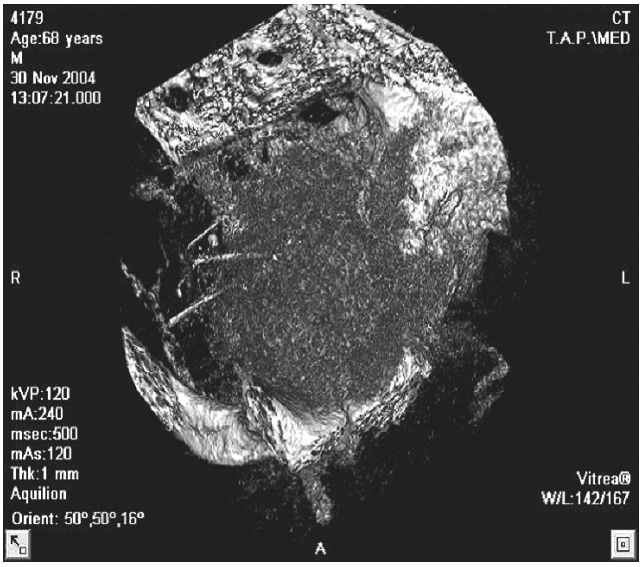

Figure 3 - First 3D- pelvic CT scan. It confirms the presence of a huge expansive solid lesion, highly vascularized, in the pelvis, diameter >8 cm., not dissociable from the left ilium and pelvic bones. The lesion, including the presence of bone spicules in the caudal portion, extended toward the rectum and infiltrated the bladder wall, incorporating the ipsilateral ureter in its left distal portion.

Table 9 - Scheme of the protocol of the RANK exon 1 mutational analysis.

Primer forward: GAGCTTGGGCACCACCTG
Primer reverse: CAAAACTCCGGCTCCTCTG

AMPLIFIED DNA: 372 bp (capital letters indicate the coding sequence of this exon)
gagcttgggcaccacctggctggcaccgccgggcccggccggaggggggcgcaggagggcggcgcgggggcaggtgcggggcgg
ggcacgggggcgggaccacacagggccgcggcagccgcgcccgctgggccacagaggccgctgaggccgcggcgcccgccagc
ctgtcccgcgccATGGCCCCGCGCGCCCGGCGGCGCCGCCCGCTGTTCGCGCTGCTGCTGCTCTG
CGCGCTGCTCGCCCGGCTGCAGGTaaggagcgcccgcgcctgccgggccgcgcggcccgacgcctcctcgggagc
cccgggaagggccggggccggcggcatcctggctcctccgccttccgagaggagccggagttttg

PCR conditions: mix 25 µl

-	Buffer 5x	5 µl
-	dNTPs 2 µM ……..	2,5 µl
-	Primer 10 µM forward…….....	1 µl
-	Primer 10 µM reverse.....	1 µl
-	DMSO.....	1,25 µl
-	Taq (Go Taq PROMEGA, USA) 5 U/ml……	0,2 µl
-	DNA (49 ng/ml)……..	1 µl
-	H_2O……..	to final volume

PCR thermal cycle: touch down

95 °C	5’	1x
95 °C	30”	8x, (-1°C at each cycle)
64 °C	30”	
72 °C	30”	
95 °C	30”	28x
56 °C	30”	
72 °C	30”	
72 °C	10’	1x

Sequence reaction: mix 9 µl

-	H_2O……..	to final volume
-	Buffer 5x…….....	1,5 µl
-	Big Dye V1.1……..….	1 µl
-	Primer 3.2 uM forward.....…	1 µl
-	PCR product……	0.5 µl

and

-	H_2O……..	to final volume
-	Buffer 5x…….....	1,5 µl
-	Big Dye V1.1……..….	1 µl
-	Primer 3.2 uM reverse.....…	1 µl
-	PCR……..	0.5 µl

mor lesion, respectively.

Genetic analysis

Since in recent studies it has been demonstrated a relatively frequent involvement of *SQSTM1/p62* gene mutations in Italian patients with sporadic and familial PDB (28-30), in the original paper by Nuzzo et al., DNA test has been performed only to search germline mutations of *SQSTM1/p62* gene in the proband (P-1) and his PDB affected daughter (P-2), with a negative result (93). However, since the proband and his daughter had a very precocious occurrence of polyostotic PDB, at age of 30 and 20 years respectively, we estimated correct to exclude/assess the possibility of a Paget-like disease.

Consequently, we performed also a genetic study in the search of germline activating mutations of *TNFRSF11A* gene (Table 9), encoding RANK, which has been reported as causal of Familial Expansile Osteolysis (FEO), Skeletal Expansile Hyperphosphatasia (ESH), and early onset PDB (EO-PDB), considered being allelic diseases (Table 10) (35-39). Again, no germline mutations were detected. Unfortunately, no tissue samples were available for this analysis.

Table 10 - FEO, ESH and EO-PDB are allelic diseases.

Type of RANK mutations	84dup18	84dup15	75dup27
Clinical Phenotype	FEO	ESH	EO-PDB

Current Therapy

Due to the persistence of the negative or unsatisfactory results, described at Table 8, the patient was included in a multicentre, Phase 2 open-study using denosumab specifically designed for patients affected by GCT, at a dose of 120 mg, SC, Q4W, with a loading dose of 120 mg, SC, at day 8 and 15 of the study. The maximum extension period allowed is 54 months, with 36 months of enrolment, 12 months of treatment and 6 months of follow up. As soon as after 15 days from the beginning of treatment, the patient achieved a weight loss of about 25 kg, a reduction of both pain and abdominal mass, a resumption of ambulation and self-stabilization of both serum Alkaline Phosphatase (after 3 months: 398 IU/L – normal range: 64-300) and MR imaging. Indeed, the last control (Figure 4), at month 6, showed no substantial changes compared to the one at month 3.
A periodically performed compilation of the Health Assessment Questionnaire for the assessment of daily capabilities and ability of the upper and lower limbs in action (disability assessed by 8 categories of activities: dressing, arising, eating, walking, hygiene, reach, grip, and common activities), showed a transition from an initial score of 3 (indicative of maximum disability), at the beginning of this treatment, to a current score of 0 (no significant disability and no need of aid), at the follow-up visits.

Discussion

The history of our patient was positive for a familial form of PDB. However, considering the following issue: 1) very early age at diagnosis (PDB-1 and PDB-2 respectively at 33 and 20 years) in comparison to what generally reported for the classical PDB (>55 years); 2) negative result of the mutational analysis of *SQSTM1/p62* and *TNFRSF11A* genes, it seems appropriate to suspect the involvement of other molecular anomalies/alterations, mutations not yet defined or currently identifiable, as also of other not identified pathways.
The treatment of GCT is problematic and more difficult could be the one of PDB-GCT that could exhibit a more severe behavior. In general, a response to corticosteroids therapy was reported in a few cases of GCT and a fairly rapid recovery of the disease was found after discontinuation of steroid therapy.
As previously reported, also in our case the continuous corticosteroids administration has helped to stabilize the disease clinically, biochemically and radiographically. Unfortunately, the lack of mass reduction and the occurrence of side effects due to use of corticosteroids, required discontinuation of treatment with relapse of pain, claudicatio of the lower left and increase of alkaline phosphatase.
Moreover, the selective arterial embolization, justified by the rich vascularization of the tumor, angiographically shown, had not cytoreductive results, while intravenous infusion of zoledronic acid resulted in a good clinical outcome in terms of response to pain control and reduction of serum alkaline phosphatase.
Currently, the patient is treated with denosumab 120 mg, SC, Q4W, (loading dose of 120 mg at day 8 and 15 of the protocol). A significant response to the drug was early evidenced and after only 15 days of treatment, the patient achieved a weight loss of 25 Kg, a reduction of both pain and abdominal mass, a complete recovery of autonomous walking and improved his daily-life relationships.

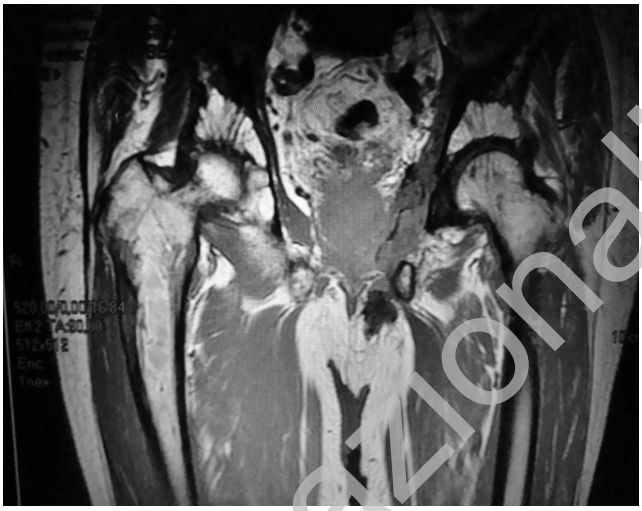

Figure 4 - Pelvic and proximal femurs MR performed after denosumab therapy. It confirms the presence of multiple osteo-structural alterations with extensive bone erosion of both iliac wings, sacral wings, L4-L5-S1 vertebral bodies, the ischiopubic left branch, right pubic bone and both proximal femurs. Extra osseous pathological tissue, predominantly at low level of the iliac muscles bilaterally, obturator and left ileopsoas muscles, is currently detectable".

Conclusions

At present, there is no standard therapy for this disease, either traditional GCT or PDB-GCT, and the treatment has to be evaluated from time to time depending on the characteristics of the tumor and patient's clinical condition.
However, the validity of a new drug such as denosumab may be clearly shown, particularly in recurrent or surgically unresectable GCT, even when associated to PDB. The validity of this drug may be also linked to its good tolerance. In fact, no patient reported significant adverse events to therapy or development of antibodies to denosumab in clinical trials. Specifically, in our patient a rapid and immediate response to treatment with tumor regression, stabilization of blood levels of alkaline phosphatase and improvement of the quality of life, with return to common-relational daily activities, have been reported.
The possible role of denosumab and other new therapeutic targets in the treatment of GCT, PDB-GCT and related disorders, is currently object of worldwide active studies.

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