Author’s response to reviews

Title: The identification of H3F3A mutation in Giant Cell Tumour of the Clivus and the histological diagnostic algorithm of other clival lesions permit the differential diagnosis in this location.

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Author’s response to reviews:

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Dear Editor,

Thank you very much for the attention given to our work and for the possibility to resubmit it. We revised and hopefully improved the manuscript, taking into account the helpful comments of the reviewers. In the preparation of the revised version of our work, all issues raised by the referees have been addressed.

Please find below point-by-point replies to the reviewers’ comments.

Looking forward to receiving your feedback,
Dear Reviewers,

Thank you for your helpful comments. We have read your suggestions carefully and revised the manuscript accordingly. For this purpose, we added two new paragraphs in the Result section, a new figure (Figure 5) and a new table (Table 1).

We hope it meets with your approval.

Please find below a point-by-point response to your concerns.

Thomas Barth (Reviewer 1):

In the MS entitled: "Histone 3.3 mutation is responsible for the rare giant cell tumor of the clivus: implication for diagnosis and treatment the authors have analyzed two giant cell tumors of the clivus regarding the mutational status of H3F3A,-B, IDH1 and-2, and ZN687. They further included immunoistochemical data regarding the mutational antibody G34w and tenascin as well as RANKL.

The MS is interesting regarding the diagnostic aspects of GCT, even though it has the character of a case description.

I can recommend publication in the case the following items are addressed:

1. Authors have to show in a meta-analysis that the clivus is really a rare location by reviewing the literature including all data possible, i.e. data from the Rizzoli Institute etc. They should show (and discuss) what are the main differential diagnosis in this region.
We thank the Reviewer for this helpful input. We added a new paragraph in the Result section titled “Systematic literature review confirms that skull GCTs are rare entities”, where we reported that skull GCTs are rare entities. As a consequence of this rare involvement of the skull, clivus as well as occipital and frontal bones result really rare locations of GCT. For this purpose, we added a new figure (Fig. 5).

In addition, we showed and discussed the main differential diagnosis of the clival region in a new Result paragraph (“Histological diagnostic algorithm permits the differential diagnosis of the main clival lesions”), and combined this information with the point no.4 raised by the Reviewer, generating a diagnostic algorithm for the different clival lesions.

2. The authors cite that they have performed tenascin stainings. However, they do not link the data to the published data, nor do they discuss or show the tenascin results. Does this help to distinguish GCT from other giant cell rich lesions or lesions in general of the clivus?

In the Discussion section, we better explained that we performed Tenascin C staining on the clival GCT biopsies because we aimed at demonstrating that this tumour shared the same histological marker than GCT of long bones, as we and others previously reported. Nevertheless, we also discussed that Tenascin C can be found in giant cell granulomas, not resulting in a specific GCT-marker.

3. The authors have analyzed H3F3-B, IDH1 and-2, and ZNF687. However, they do not discuss why they have done this and what are the results regarding the sequencing of these regions? What is the rational for this approach, does this help to exclude other giant cell rich tumors, further sarcomas?

As kindly suggested by both Reviewers, we clarified in the Discussion section the reason why we examined the coding regions of these genes. Though rarely, H3F3B, IDH1 and IDH2 have been linked to GCT development, and we identified ZNF687 as gene responsible for GCT degeneration of pagetic bones. Considering the frequent skull involvement in Paget’s disease of bone as well as the giant cells observed in the tumour sample of patient 1, we thought we’d
analyse also this gene. In the Result, we also pointed out that no mutation was found in these genes. In this study, we did not examine other giant cell rich sarcomas. However, to support the utility of H3F3A molecular screening as a diagnostic tool in GCT, we reported recent studies demonstrating that H3.3 alterations are only found in GCT.

4. I suggest to generate to an diagnostic algorithm including histology for the most frequent lesions of the clivus region including metastases, chordomas, further types of sarcomas etc.

We added a new paragraph in the Result section and a table (Table 1), describing the main differential diagnosis of lesions affecting the clivus, highlighting their histological features and distinctive staining markers. Matching data coming from this table may be useful in the differential diagnosis of these lesions. Regarding metastases at clivus, we were not able to find a distinctive immunohistological pattern according to the markers identified for the primary lesions of the clivus. We highlighted that, for a proper diagnosis, metastasis tissues should be stained with markers specific for their primary tumours.

5. What is the clinical course of the disease in the two patients? Are they free of disease? Further therapy?

For what concerns the patient 1, we emphasised that, so far in 2016, she is still suffering of worsening headache, tongue paralysis and dysphagia because of tumour recurrence, and surgery has been planned again.

Regarding patient 2, we added further clinical information in the first paragraph of the Result section about symptoms, therapy and relapse.

6. Change the title. The authors do not show that the mutation is responsible for this tumor. They detect the mutation in the tumor, however, this is no proof of principle such as a knock-out/-in experiment. A suggestions is: GCT of the clivus as a rare entity in this location
We welcomed Reviewer’s suggestion to change the title and we did as follows: “The identification of H3F3A mutation in Giant Cell Tumour of the Clivus and the histological diagnostic algorithm of other clival lesions permit the differential diagnosis in this location.” With this title, we would like to emphasise that H3F3A mutational screening is an essential diagnostic tool to distinguish GCT from morphologically overlapping giant cell-rich tumours (namely giant cell granuloma, giant cell-rich osteosarcoma, pagetic osteosarcoma, aneurysmal bone cyst, etc…). On the other hand, for clival tumours not characterized by giant cells, the histology-based diagnostic algorithm that we generated may be useful for differential diagnostic purposes in histologically ambiguous cases.

Corinne Bouvier, Ph.D, M.D (Reviewer 2):

Giant Cell Tumours of Bone (GCT) are locally aggressive primary bone tumours that mainly developed in long bones more infrequently in skull. Recently recurrent driver somatic mutations in the H3F3A gene have been found in more than 90% of cases in GCT. Mutational analysis has become a diagnostic tool for GCT as well as immunohistochemistry with antibody anti-H3F3A G34W directed against the main mutation. The paper reports 2 cases of GCT arising from the Clivus with genetics data. It is a very exceptional condition often leading to an incomplete surgery. The accuracy of diagnosis is important since systemic treatment such as Denosumab could be used with success for CGT. The aim of the study was to describe the genetics background of these exceptional tumors searching for H3F3A, H3F3B, IDH1, IDH2 and ZNF687 gene mutations. The team has an expertise in genetics studies and recently identified a germline mutation in ZNF687 in GCT occurring in Paget's disease. Very few primitive GCT of the Clivus have been reported (15 cases) and for very few molecular study was done.

Major concerns:

2 additional GCT have been recently reported by F. Amary et al. in Am J Surg Pathol 2017 harboring H3.3 G34W mutation. This has to be added in the discussion and bibliography.
We added in the Discussion section (and in the Bibliography) that, since the identification of H3F3A alterations in GCT samples from Behjati and collaborators in 2013, several other groups confirmed the causality of H3.3 mutations in this tumour. We mentioned them, including the one suggested by the Reviewer, and also highlighted that the p.Gly34Trp is the most commonly found mutation.

Identical mutation seemed to be present in GCT of the Clivus as in their long bones counterpart but this statement has to be discussed in the light of the small number of the tumours investigated.

We clarified in the Discussion section that, even though only two cases of GCT affecting the clivus were genetically defined by mutation in the H3F3A gene, this result links the Clival GCT to the GCT of long bones.

The search for ZNF687 mutation should be explained: systematically research or because the patients had clinical criteria for a Paget's disease? It seems that for the patient number 1 on microscopic section (figure 1) the giant cells have very numerous nuclei a special histological feature the authors described in their paper dealing with GCT developped in Paget's disease. Skull is also a bone frequently affected by Paget's disease. So a comment must be added about this issue.

As kindly suggested by both Reviewers, we explained in the Discussion section the reason why we examined the coding region of ZNF687. We recently identified it as gene responsible for GCT degeneration of pagetic bones. Considering the frequent skull involvement in Paget’s disease of bone as well as the giant cells observed in the tumour sample of patient 1, we thought we’d analyse also this gene.
Minor concerns:

No information is provided about the quality of the resection for the first tumour of the patient number 1. So it is inappropriate to speak of a recurrence one month later. The discussion about the prognostic value of the expression of the tenascin C is questionable in this context.

We agree with the Reviewer’s concern and then removed the statement that the patient underwent tumour recurrence after 1 month from the Discussion. Currently, we only refer to the last recurrence after 2 years from the intervention. Moreover, we better explained that we performed Tenascin C staining on the clival GCT biopsies because we aimed at demonstrating that this tumour shared the same histological marker than GCT of long bones.

In the legend of the figure 2 informations have to be added: MRI images of the first patient? of the recurrence ???

As has been highlighted by the Reviewer, we missed some information and we proceeded to add that the MRI refers to the recurrent Clival GCT of patient 1.