Author’s response to reviews

Title: A case of Molecularly Profiled Extraneural Medulloblastoma Metastases in a Child

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Version: 2 Date: 20 Aug 2017

Author’s response to reviews:

Dear Editor,

We would like to thank you and the reviewers for their valuable comments. We revised the manuscript accordingly. Furthermore, we performed a methylation testing to strength our data with our collaborator at NYU and included him as a co-author.

Best,

Malak Abedalthagafi

Reviewer reports:

Stephen Gilheeney (Reviewer 1): As a single patient case report, there are no "controls" to comment on.

The authors present a single patient case report of the molecular genetics regarding one patient who developed extra-neural recurrence of medulloblastoma. The timeline of the case is
presented well, and the methods for assessing the tumor are adequately described. It is difficult
to make conclusions on the basis of one case without becoming strident in one's commentary.
The authors manage to avoid this. I find the manuscript acceptable for publication as a case
report or a letter.

We would like to thank reviewer 1 for his positive feedback.

Yasmin N. Khakoo (Reviewer 2):
A. Title: Fine
B. Abstract: Fine
C. Background: Fine
D. Case presentation: Shorten by 25%
We did shorten the case presentation.
1. What is the dose of radiation? Now included
2. The authors should specify what the chemotherapy regimen is based upon. Now included.

E. Discussion: Also needs to be shortened. We were not sure what exactly needed to be
shortened. Our discussion is very concise and up to date (including methylation references
now as it was suggested by another reviewer)
1. Should include the word “historically” in the first sentence 2. The authors should clearly
specify the prognosis of the 4 distinct subtypes of medulloblastoma 3. Now included with the
new references in the discussion.

F. Figures: Only need one or two MRI scans. Remove Figure 2. We removed it

G. References: Reference #4 is incorrect. Otherwise, the references are current. Authors should
also reference Young et al PMID 25504865. There is a new classification of 7 different
subtypes. We corrected the references and included more up to data study from Sick kids
group ( ref 5)
Maria A. Lastowska (Reviewer 3): The paper presents a case report describing extraneural metastases in a child with medulloblastoma. Since this is a rare clinical manifestation of the disease, molecular characteristic of tumour warrants particular attention.

The reviewer has the following concerns with the manuscript:

1) It is unclear what was the basis for diagnosis of original tumour since no histopathological examination results were presented in the case presentation section. We now included it in the case presentation and in the histological sections.

Diagnosis of 'medulloblastoma with classic morphology' and immunohistochemistry results refer to mandibular sample only, is it right? We now clarify that diagnosis was made in both primary and metastatic tumors by morphology and IHC.

2) Molecular investigation of metastatic tumour included next generation targeted exome sequencing.

In view of the reviewer, the results do not allow for classification of tumour into one out of four established groups of medulloblastoma. The plot in Figure 4 does not support the presented in the text results concerning monosomies, with exception of chromosome 3. Amplification of ETV1 and SOX9 is not indicated on the plot. (Included now) Therefore, the suggestion by the authors that tumor belongs to group 4 is at least speculative. We now included copy number data from methylation assay and we also perform a methylation assay which subs classify the MB to group 3 based on German Database.

Also, from the methodological point several questions arise:

a) It is not clear what genes were included into oncopanel used in the study. Please provide the link to the list of analyzed genes and information what was the criteria for their selection. We now included a ref 4 which describe the method in depth and list the genes list.

b) In molecular profiling of tumours the "gold standard" is to use matched patient's germline DNA to establish which abnormalities detected in tumours samples are somatic and are relevant to the oncogenic processes. Did the authors taken into account the patient's germinal material for the identification of somatic mutation?

Oncopanel (our NGS targeted test at Brigham and Women’s Hospital and Dana Farber cancer institute) is well-validated assay in the literatures with over 12000 samples tested up to date. The
pipeline of analysis takes in account the lack of germline. Recently, the analysis of >200 pediatric brain tumors without germline testing was published at Neuro-Onc (ref 4)

c) Taken into consideration that The Association for Molecular Pathology and College of American Pathologists recommend a minimal depth of coverage>250 reads per tested amplicon or target for somatic variant detection (http://dx.doi.org/10.1016/j.jmoldx.2017.01.011), obtained in the study NGS data carry increased risk of possibility of false-positive or false-negative results.

Please specify what was the pipeline of NGS data analysis. Supplementing this information is particularly important as the author's pointed that 'No notable somatic mutation were detected'.

Therefore, the absence of mutation in tumour sample may be somehow surprising. Do the authors have hypothesis explaining these results?

No we didn’t hypothesis it. This is output from the oncopanel pipeline and after the review of two molecular pathologists for the 4 tires mutations in oncopanel reporting system. Ref 4

Summarising this point, the reviewer strongly suggests an introduction of additional relevant method (e.g. methylation profiling) for identification of established molecular type of medulloblastoma in the presented case. We perform now methylation testing and added the relevant references.

As it stands, the title of this paper is overstated, bearing in mind obtained molecular results. The authors themselves underline in the discussion an importance of molecular group detection and this is not supported by presented here outcome of investigation. We included that in the discussion with ref 5,6.

3) Molecular analysis revealed amplification of MYCN, but in the abstract and in the description to Figure 4 MYC amplification is presented. Please clarify this discrepancy. Sorry for the discrepancy, we now fixed it.

4) References cited throughout the text do not correspond to the numbers in the list of references. We fixed it

5) A statement that "group 3 and group 4 tumours recur almost exclusively with extraneural metastases"... is not true, since metastases occur also within central nervous system. We deleted it.
A few minor comments are:

- Figure 2A is very distorted; also introduction of the arrows showing described features would be helpful for the readers. We fixed it.

- The name of gene should be written in italics. Corrected

- Irregularities in writing of Medulloblastoma or medulloblastoma. We fixed it all as medulloblastoma