Author’s response to reviews

**Title:** Comprehensive chromosomal aberrations in a case of a patient with TCF3-HLF-positive BCP-ALL

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Author’s response to reviews:

Lublin March 10th 2020

Dear Editor-in-Chief of the BMC Medical Genomics,

Please find enclosed the revised manuscript MGNM-D-20-00025: “Comprehensive chromosomal aberrations in a case of a patient with TCF3-HLF-positive BCP-ALL” by Monika Lejman, et al., to be submitted as a Case Report to the BMC Medical Genomics for consideration of publication.

All co-authors have seen and agree with the contents of the manuscript and declare no conflict of interest. We certify that the submission is original work and is not under review at any other publication.

As compared to the first submission, the revised version of the manuscript contains additional details prepared according to Reviewers comments (Figure 1, Figure 2, Figure 4). The results were also described in more detail.

We believe that our findings could be of interest to the readers of the BMC Medical Genomics, because the raised issues still are very popular among clinicians. We hope that the editorial board will agree on the interest of this study.

I would like to thank you for your comments.

Below I indicate the revisions, which were made according to Reviewer’s comments. The changes are highlighted.
Reviewer #1:

This report describe a rare patient with a deletion of a long arm fragment of chromosome 13 (13q12.2-q21.1) and co-existing TCF3-HLF fusion. However, this report only shows a correlation without valid evidence. The authors’ response does not reply to this question, I suggest reject this manuscript.

Revision and my comment

Our patient had a lot of clinical complications during the first line of therapy and drug resistance occurred in relapse treatment. Given the fact that we are dealing with an extremely rare case, performing additional analyses would be an undeniable advantage of our work. Unfortunately, the number of samples collected from the patient was not sufficient for further analyses, including whole genomic sequencing. The correct karyotype of patient’s parents does not indicate the necessity for additional analysis of their genetic material.

Watanabe et al. suggested that TCF3-HLF fusion may be accompanied by other genetic changes. Among the most commonly described changes co-occurring with the TCF3-HLF fusion are alterations in the RAS signaling pathway genes (NRAS, KRAS), as well as in the VPREB1 and BTG1 genes. In our case, no changes in any of these genes were observed. However, the PAX5 deletion associated with the TCF3-HLF fusion was found both in our patient and in the previously described cases. The 13q deletion was observed in the patient's genetic material collected at the time of diagnosis, but this change was not found in the material collected at relapse, which indicates the clonal evolution of cancer cells. The loss of the RB1 gene and coexisting genes in deleted fragment (SERP2, POU4F1, has mir-15a, OLFM4, DLEU7, FOXO1, DACH1, TRIM13, LHFP, KCNRTG, KLFS, LCP1, ST13P4, INT5S6, DLEU2, DLEU1, DGKH, RB1, ELF1, mir-16-1) in a patient with TCF3-HLF fusion was described by us for the first time.

In our case, we are dealing with two rare genetic alterations: TCF3-HLF fusion and 13q deletion. In ALL cases, RB1 gene deletion is observed more often than loss of a fragment of 13q. Moreover, 13q deletions are mostly described as secondary changes. Downregulated expression of RB1 gene is associated with poor response to treatment. Overall survival in patients with RB1 gene loss and in patients with RB1 gene deletion coexisting with additional aberrations within 13q is similar, but patients with additional aberrations within 13q demonstrate shorter events-free survival. These findings indicate that cellular reprogramming might be associated with a very aggressive disease course and resistance to treatment observed in our case.

Additional genetic analysis allows to identify activating mutations of genes associated with drug resistance. In case of TCF3-HLF-positive patients, the biggest challenge is to identify co-occurring genetic alterations and to define its impact on outcome. Due to rarity of TCF3-HLF fusion among BCP-ALL cases, further analysis of genetic changes is necessary to personalize treatment. Moreover, identifying additional aberrations in TCF3-HLF-positive might be the clue to development a new targeted therapy or new treatment protocol to improve the outcome of ALL patients.
Our results, illustrating comprehensive changes in the karyotype and clonal evolution of cancer cells, call into question the effectiveness of experimental therapy. The cumulative effect of the described changes has both diagnostic and therapeutic implications. The patient was treated in accordance with the ALLIC-BFM 2009 protocol, according to which the stratification of patients is based on a karyotype analysis and the presence of BCR/ABL1, KMT2A, ETV6/RUNX1 rearrangements. The updated version of this protocol (AIEOP-BFM ALL 2017), introduced in Poland in 2018, imposes the obligation to diagnose TCF3-HLF fusion. The described case is another step to understand the complexity of the impact of other changes on the outcome of patients with established TCF3-HLF fusion. Additional research is necessary to determine the mechanism by which comprehensive changes can affect the outcome.


Reviewer # 2:

Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format. Please overwrite this text when adding your comments to the authors.

Revision and my comment

All comments were provided according to the Reviewers requests. The manuscript was reorganized.

Reviewer # 4:

The authors have taken action on many of the comments and suggestions raised. As such, the amended manuscript flows nicely when read and the text and figures are markedly improved.

However, there are some revisions that are only partially complete, or new additions that have raised further issues. These are all readily addressed. For ease of reading, I've included complete reference to my original comments and the abridged associated revisions of the authors.

Original Comment 5:
If the patient karyotype was determined by GTG band staining, the authors should show this as a figure to confirm normality. If the statement of a normal karyotype is solely based on the observed FISH results, this would only be suggestive of a lack of disruption at these select loci, and not overall patient normalcy.

Author Revision:

… … … The absence of aberrations of 13q in the karyotype analysis indicates that only cells with a normal karyotype have grown in cell culture. … … …

New Comment 1:

This sentence needs to be incorporated into the manuscript text, as it's an interesting observation. (One would assume that these cells would grow more readily in culture?)

Revision and my comment

This sentence was incorporated into the manuscript text. (Case presentation section, line 99-101, page 6).

Original Comment 7:

(Lines 88 to 98) Out of all the retrospective tests that could be applied, why specifically was TCF3-HLF FISH performed? If it was part of a battery of FISH tests, what other assessments were attempted?

Author Revision:

Performing additional analyses of patient's genetic material is a routine procedure used in patients with short survival in whom a very aggressive disease course and resistance to treatment were observed. Following the data discussing cases of patients with a similar course of the disease, we used probes for TCF3-HLF.

New Comment 2:

A revised form of these sentences needs to be incorporated into the manuscript text, to assist the reader in understanding why specifically TCF3-HLF FISH was undertaken.

Revision and my comment
Original Comment 8:

(Lines 91 to 96) Whilst the microarray was applied to samples at diagnosis and at relapse, TCF3-HLF FISH (and GTG band staining) only seems to have been applied at diagnosis. Why is this? Further, it would also be more comprehensive for the authors to show the effects of genomic disruption of RB1, PAX5, CDKN2A, CDKN2B and NOTCH1 through either qRT-PCR methods or IHC. I.e. show an effect on each respective gene at either the transcriptional or translational level. qRT-PCR would also provide good confirmation of the TCF3-HLF FISH result, both at diagnosis and at relapse.

Author Revision:

… … … Unfortunately, the amount of protected samples from the patient was not enough for further tests, including RT-PCR.

New Comment 3:

This statement should also be included in the manuscript text as a rationale as to why RT-PCR was not undertaken.

Revision and my comment

This sentence was incorporated into the manuscript text. (Case presentation section, line 104-106, page 6).

Original Comment 13:

Figures: (1A) There is no description of the figure labelling or figure colours in the legend.

Author Revision:

The figure was improved and the description has been provided (Figure 1 and Figure 2 … … …

New Comment 4:
The legends for revised Figures 1 and 2 need to state the probe colour scheme, with additional annotation (e.g. arrows, etc.) to assist with FISH image interpretation for all panels.

Revision and my comment

Figures 1 and 2 were improved.

Original Comment 14:

(1B) Some of the details of this figure legend would be better served in the main body of the text. The figure itself is of very poor quality, is not well annotated and is barely legible.

Author Revision:

The figure was improved (Figure 4 … … …

New Comment 5:

The "lightning bolts" need to be defined in the figure legend. An indication also needs to be made in the figure that there are additional upstream / downstream exons not graphically represented for clarity. I would suggest a "fade out", dotted lines, or similar.

Revision and my comment

The “lightning bolt” was defined in the figure legend and the figure was improved. Following Reviewer’s suggestion, we added the sentence indicating that there are additional exons not graphically represented.

Sincerely yours,

Monika Lejman on behalf of the authors.

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