Reviewer’s report

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

Version: 1 Date: 05 Mar 2020

Reviewer: James Blackburn

Reviewer's report:

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:

(Lines 73 and 74) 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?)

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.

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.

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.

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.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

Not relevant to this manuscript

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