Reviewer's report

Title: Detection of ALK Fusion Transcripts in FFPE Lung Cancer Samples by NanoString Technology

Version: 0 Date: 15 Apr 2017

Reviewer: Miaozhu Li

Reviewer's report:

Evangelista et al perform a validation study of NanoString ALK fusions in a cohort of NSCLC patients. They validate the sensitivity of the NanoString technology, and optimize the method with initial input of 100 ng of RNA to detect ALK fusion in a real clinical setting. The main contribution of this study is to identify the alternative method for detecting ALK fusions is low quality and quantity RNA in lung biopsies of FFPE specimens. The revised manuscript has been greatly improved in its content as well as its English grammar. A few things need to be addressed by the authors to enhance their manuscript.

1. It would be better the author add the limitations of the NanoString fusion assay in the discussion, especially they list NanoString requires higher hardware & software cost in comparison to other methodologies.

2. Toni-Maree Rogers et al has performed a comparison of FISH testing, NanoString, Agena LungFusion panel and ThermoFisher NGS in 51 clinical specimens on Sci Rep. 2017. They also report the high agreement of NanoString (with 94% positive agreement and 97% negative agreement). It would be more appropriate to reference their study. It seems NSG is more sensitive than NanoString in low input RNA. The input requirements for different technologies should be compared as well.

3. There is no specific threshold at which a fusion is called positive for NanoString fusion assay. The criteria established by Lira et al. (2013) was 2 and the authors used the fusion threshold at 20 counts after normalization. So it might be not appropriate to claim NanoString is the best potion in terms of interpretation of the results, when there is no specific analysis pipeline for fusion detection, or a threshold setting value for this method.
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.

I am able to assess the statistics

Quality of written English
Please indicate the quality of language in the manuscript:

Acceptable

Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?
If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests.

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal.