Reviewer’s report

Title: Accurate Detection of KRAS, NRAS and BRAF Mutations in Metastatic Colorectal Cancers by Bridged Nucleic Acid-Clamp Real-Time PCR

Version: 2 Date: 24 Sep 2019

Reviewer: Takao Iida

Reviewer's report:

The authors did not delete "designed with TaqMan probe" in the METHODS of revised manuscript. The revision should be confirmed before it is submitted.

According to the authors' responses to the comments of Reviewer #3, to detect of mutations in KRAS, NRAS and BRAF genes accurately, both the one-step real-time PCR method using BNA-probe and subsequent Sanger sequencing were needed in the manuscript. But there are concerns based on the above in the manuscript.

1. In the sections of ABSTRACT, BACKGROUND and METHODS, BNA-clamp PCR was mentioned as bridged nucleic acid-clamp real-time PCR method by the BNA Real-time PCR Mutation Detection Kit Extended RAS. On the other hand, in the section of RESULTS, it was described as combination of the real-time PCR method and subsequent Sanger sequencing. Furthermore, the former and the latter coexist in the DISCUSSION and the CONCLUSIONS. It is inconsistent in the manuscript.

2. In the page 13, 14 and 17, turnaround time and running cost of the BNA Real-time PCR Mutation Detection Kit Extended RAS only were mentioned. The authors need to describe these of the method combined the real-time PCR and subsequent Sanger sequencing.

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

**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/](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