Reviewer's report

Title: Can we accurately report PTEN status in advanced colorectal cancer?

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Reviewer: Francesca Molinari

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In the manuscript "Can we accurately report PTEN status in advanced colorectal cancer?" by C. Hocking, the Authors evaluated PTEN copy number variation (by Taqman assay) and protein expression (by IHC) in 56 colorectal cancer specimens in order to analyse the inter-observer variability of IHC evaluations and the concordance of PTEN status between the two methods used to analyse PTEN. I would like to thank the authors to have taken into account my suggestions and to have reply to the majority of my requests. I think that the revised version of the manuscript is now very much improved especially the discussion section. I still have some doubts on some points that I hope you can solve.

Major Compulsory Revisions

1. An explanation on how the PTEN copy number of the HT-29 was known have been added in the text. The same has to be done for the LIM2405 and LIM1899 cell lines as these cell lines, together with the HT-29 cell lines, have been used to validate the PCR assay and so you have to be sure that the LIM2405 and LIM1899 contain 1 and 2 copies of the PTEN gene respectively. Moreover, the LIM1899 cell line has been used as calibrator and so the PTEN copy number of this cell line, and also of the LIM2405, must have been established also by other methods (in the literature or by your previous studies).

2. The authors must include in the material and methods section that they use as calibrator for CNV analysis the cell line LIM1899, on pending demonstration of the presence of 2 copies in the cells with another methodology (See comment above). This information is important to understand how the calculations of 2-##Ct has been done.

3. Regarding the five specimens in which only the pathologist JC didn’t find tumor cells, I return on the point that these cases have to be excluded from the analysis or re-evaluated for the presence/absence of tumor cells. You have replied me that the IHC concordance rate includes the cases where no tumor was identified but in the material and methods section is written that the cases where selected on the basis of the presence of tumor cells. Moreover it’s not possible that two pathologists, on the same section, found different things as regard the presence or absence of tumor cells. If the pathologists have evaluated different sections, it could be possible that one pathologist found tumor cells and the other one not on a different slide, but in this case, you cannot use your results for inter-observer variability assessment, because, for inter-observer variability studies, you have to
evaluate the same slides. Please, clarify this point.

Minor Essential Revisions

1. Introduction, 1° paragraph: the sentence “In addition to KRAS, mutation of genes involved in downstream EGFR signalling pathways Ras/Raf/MAPK and PIK3CA/AKT also confer resistance to anti-EGFR MoAbs” has to be changed in “has been proposed to confer” as the role of BRAF, PIK3CA and PTEN has not been validated yet.

2. Introduction, 1° paragraph: in the sentence “Specifically, mutations in BRAF and NRAS genes…”, remove NRAS (as you have added in the text above together with KRAS) and you can replace with PIK3CA gene for which has been proposed also a role as predictive marker.

3. Introduction, 4° paragraph: in the sentence “other groups have assessed PTEN loss using FISH etc…) do you mean other groups studying the PTEN predictive value? If yes please add in the text. Moreover by reading this sentence it seems that with FISH analysis you can detect both PTEN loss, mutation and methylation, so this sentence has to be rewritten better.

4. Introduction, 5° paragraph: I think there is a typing error: “taqMan and ref”

5. Results, last paragraph: in the description of how many cases have PTEN allelic loss in Taqman and in IHC, please add also that 15 cases which do not have PTEN loss both in IHC and Taqman because this information cannot be obtained by the present description.

6. Discussion: in the first sentence you would add the inter-observer variability as the purpose of the study is in first to evaluate the interobserver variability and next the comparison of PTEN status with two different methodologies.

7. Discussion, 2° paragraph: the percentage of 32% is wrong: 7 (IHC positive expression)/17 (allelic loss) = 41% 

8. Discussion 5° paragraph: thank you for adding the reference of Sangale and colleagues. As they found 100% concordance between 3 pathologists you have to discuss your discordant results giving possible explanation of this discordance.

For all these reasons the paper can be accepted pending revision.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interest