Reviewer's report

Title: A six gene panel for the molecular detection of circulating tumor cells in the blood of female cancer patients

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Reviewer: Robert Brown

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The manuscript by Obermayr et al describes the analysis of the potential of a 6 gene panel to detect by RT-PCR of RNA from blood the presence of circulating tumour cells (CTCs) in a subset of gynaecological tumours. This is a potentially important and clinically relevant question, although it should be noted that, as opposed to immunohistochemical markers, RT-PCR of target genes of CTCs will not allow their isolation, but rather their potential quantification. Nevertheless, given the potential prognostic and predictive value of levels of CTCs this is of importance.

Discretionary Revisions

1. The comment on page 13 that “Due to technical constraints and to a 10% reduction of the granted fund we decided not to analyze tissue samples from cervical carcinoma patients.” is not really required, although it is a pity that analysis of these samples could not be included.

2. At times the English can be difficult to follow or is not clear. Some editing of the English would help.

Minor Essential Revisions

2. There is reference to a Table 6 on page 15, but the manuscript only contained up to Table 2.

3. Three standard deviations from the mean expression in healthy control blood samples was used to select the 6 gene panel. Presumably this is also used to define positivity in Table 2, although this was not particularly clear in the legend.

4. The sensitivity measures of the assay may more usefully come earlier in the results section.

5. In the conclusions the authors should state that the comparison is with mRNA expression of hMAM or EpCAM alone.

Major Compulsory Revisions

6. Given the variable success in the 6 gene panel in detecting over-expression in cancer patients (19-81% depending on tumour type) the title should be qualified to something equivalent to “Assessment of 6-gene panel...”. At present it sounds
as though the panel detects CTCs in all female cancer patients with CTCs, which has not really been demonstrated in the study.

7. The exact reasons for choosing the 6 genes are difficult to extract from the paper. A schematic showing the route to how these were chosen would be useful to the reader.

8. Tumour derived RNA has been previously reported to be detected in cancer patient serum and plasma. Is there the possibility that the over-expression detected could be derived from plasma/serum RNA present at start of RNA extraction of patient samples rather than CTCs?

9. Given that 15% of samples did not have good quality RNA (page 14), were these included in subsequent studies? Given that the authors have importantly measured the quality of the samples, poor quality samples should be removed from the study.

10. The data for expression of the 96 genes in tumour tissue, especially the frequency of detecting expression in different tumour types, described on page 15 should be shown.

11. Are PLEKHC1 and SGCB detected in all ovarian tumours?

12. Is there heterogeneity in levels of RNA of these genes?

13. In the section on gene expression in tumours on page 15 are the authors referring to over-expression of these genes and, if so, relative to what?

14. It would be useful to report the sensitivity/specificity of the panel for detecting cancer in these patients. Ideally this should be compared to other methods of detecting CTCs in this group of patients such as Veridex CellSearch test using EpCAM.

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

**Quality of written English:** Needs some language corrections before being published

**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 interests