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: Christoph Klein

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The authors have designed a stepwise approach to identify new markers for the detection of circulating tumour cells in the blood of patients with advanced and primary breast, ovarian, cervical or endometrial cancer. They first analyzed the gene expression profiles in 38 cancer cell lines blood samples of healthy donors. Genes differentially expressed in both groups were analyzed by RT-qPCR to confirm their differential expression to another, independent control group of healthy donors. The expression of the validated target genes was measured by the same RT-qPCR in blood and tissue samples of additional patients with primary breast, ovarian or endometrial cancer and also in blood samples of healthy women. By this approach six genes were found to be over-expressed in the blood of the cancer patients. This panel is suggested to outperform previous markers for the detection of circulating tumour cells in the blood.

Major compulsory revisions:

For a critical evaluation, the reviewer misses fundamental information on the procedure and the patients.

1. It is not stated when the blood samples were taken, i.e. whether or not primary tumours or residual tumour masses were present at sampling. For any use as monitoring assay or as assay for early detection, however, it is essential that the samples are taken after removal of tumour masses detectable by imaging techniques or before positive mammography.

2. The term “advanced disease” is poorly defined. Is it locally advanced disease or manifest metastasis? Is it lymph node metastasis or distant visceral, bone marrow, brain metastasis?

3. Patient selection is obscure and appears random, which impedes a critical evaluation and leads the authors to draw logically awkward conclusions. For example, the statement “We found that CCNE2, DKFZp762E1312, EMP2, and SLC6A8 gene expression in tumor tissues reflects the tumor stage rather than the cancer type, as more ovarian cancer patients than breast or endometrial cancer patients were RT-qPCR positive”, cannot be drawn. Table 1 demonstrates that from both cancer types incomparable stages were included. Therefore, to address any meaningful difference between tumour types, numbers must be increased and be comparable. Any conclusion can only be made within
4. Table 1 contains additional mysteries. For example, what is meant by “advanced” stage 1 disease and what is “primary” stage 4 disease? Why is the distinction between “primary” and advanced” disease only applied to breast cancer and not to the other cancer types?

5. Some details of the approach deserve further experiments and discussion. For example the initial screen used Ficoll for blood of healthy donors. Subsequently, the authors use OncoQuick to prepare samples without any explanation why this should be done. Ficoll has a density of 1.077 g/ml while the density of OncoQuick must be substantially lower as only thrombocytes but no leukocytes are held in the interphase (manufacturer’s information). Thrombocyte density is about 1.03 – 1.06 g/ml. Indeed, the authors observe that previously validated markers for CTC detection are of little use after OncoQuick. However, also the six marker panel does not entirely convince as, unlike before, several genes lose specificity. For example, 356 genes differentially expressed in cancer cell lines compared to blood of healthy donors were initially discovered (Ficoll) and 93 of them were chosen for further analysis after RT-qPCR. But more than 50% of them (55 out of 93) showed no differential expression in blood samples from cancer patients compared to healthy controls (OncoQuick). Two, possible reasons may be causative for the largely disappointing results. (i) OncoQuick may enrich cells in healthy donors which express the genes of the 356/93-gene lists or (ii) OncoQuick may in fact deplete cancer cells with high expression of genes from the 356/93-gene list. Therefore, the authors should repeat the analysis by loading a sufficient number of blood samples of healthy and tumour donors onto Ficoll and OncoQuick (50% each for direct comparison). Thereby, consistency with the initial approach can be obtained and the role of density gradients can be assessed. In addition, the 356-gene list must be provided (i.e. microarray data must be deposited into database).

6. It is unclear, how the analysis of the primary tumour samples contributed to the selection of the six genes.

7. Table 2 must be improved. The meaning of the numbers is not explained – percent values or real numbers?

8. Finally, the experiments do not explain why the marker panel should be useful for the detection of early breast cancer as stated in the discussion. The marker panel was selected after positive findings in metastatic breast cancer patients.

Level of interest: An article of importance in its field

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.