Author's response to reviews

Title: Gene expression variation to predict 10-year survival in lymph-node-negative breast cancer

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Author's response to reviews: see over
Dear dr Pafitis,

We were very pleased that our manuscript, “Gene expression variation to predict 10-year survival in lymph-node-negative breast cancer” has been accepted for publication in your journal.

Your instructions to submit the revised manuscript and cover letter were carefully followed. We hope that the revised manuscript is now acceptable for publication in BMC Cancer.

Our specific responses to the reviewers’ comments are listed on the following page.

Thank you very much.

Sincerely,

Elin Karlsson
The 51 gene signature showed good predictive value when tested on another publicly available data set published by van’t Veer at al., containing lymph node negative breast cancer patients, whereas the predictive value was poor when tested on the Wang data set. The 70-gene signature identified by van’t Veer et al. was later validated in a larger tumour set including both lymph node negative and node positive breast cancer patients. Did the authors also attempt to evaluate the signature in any other data set, where also lymph node positive patients were included?

-No, since our study is on node negative breast tumours, we concentrated our evaluation on two well recited articles. Of course, it would be interesting to test whether the gene set has prognostic qualities in node positive tumours as well. We might test the gene list in a larger set of tumours in the future (revision on page 16).

Perhaps the authors want to discuss the limitations of using this type of selected material for analysis of gene expression profiles.

-A paragraph is added on page 16.

On page 5, in the materials and methods section, it is stated that the authors accepted RNA specimens with 28S/18S ratio >1.0 and RIN>6.7. On what basis were these thresholds accepted? I believe it is more common to use higher threshold values in the order of 28S/18S ratio >2.0 and RIN>8. Perhaps sub-optimal quality of RNA could explain the failure to verify the microarray findings for 4 out of the 14 genes analysed by RT-PCR? Please correct the typo 28S/16S.

-RIN values ≥ 6 are recommended to be used for gene expression analysis in fresh frozen breast cancer tissue (Strand, et al. 2007). The RNA quality might be the reason why we could not verify all genes from the microarray experiment (revision on page 12). The typo is also corrected at page 5.

The authors did not find any overlapping genes with the van’t Veer and Wang signatures, respectively. Some of the genes of these signatures, however, appear to be involved in similar biological pathways, which perhaps could be mentioned. This has been found in other publications, where a low degree of overlap with other gene expression signatures was reported.

-This is mentioned on page 14.

The discussion section is slightly over-detailed and could probably be modified and shortened; for example some sentences repeating facts provided in the results section could be removed.

-In order to shorten the discussion section and avoid repetition, one paragraph in the result section concerning post surgical treatment is removed, and parts of the discussion section is modified.

It is not clear if the present microarray data will be made publicly available.

- It will be at the Gene Expression Omnibus repository (page 6).