Author's response to reviews

Title: Comparison of molecular and immunocytochemical methods for detection of disseminated tumor cells in bone marrow from early breast cancer patients

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Author's response to reviews: see over
Dear Editor,

Thank you for considering a revised version of our manuscript entitled:

“Comparison of molecular and immunocytochemical methods for detection of disseminated tumor cells in bone marrow from early breast cancer patients”

for publication in BMC Cancer.

As requested, we have addressed the reviewers' comments and incorporated the changes into a new version of the manuscript. The changes have been highlighted in the revised version of the manuscript, and point-by-point answers to the reviewers' comments are given below (Italic).

We hope that you find our revised manuscript suited for publication in BMC Cancer.

Yours sincerely,

Bjørnar Gilje (MD)
**Referee #1:**
Authors discuss important topic related to comparison two methods for detection of disseminated tumor cells in bone marrow of primary breast cancer patients. Manuscript is clearly and concisely written and could be published in BMC Cancer.

**Major Compulsory Revisions**
None

**Minor essential revisions:**
Authors should discuss higher detection rate in samples BM3 and 4, compared to BM1 and 2.

**BM3 and BM4 were only collected from the patients with a positive BM2 sample. Thus, if the patients remained DTC positive, 100% of the BM3 and BM4 samples should test positive. As we find that by ICC, only 14.3% (BM3) and 16.7% (BM4) of the patients have a positive DTC status, this indicates that most of the patients have changed from DTC-positive in BM2 to DTC negative in BM3 and BM4.**

We have added the following in the manuscript:

“It is important to note that BM3 and BM4 have a higher frequency of positive samples because these samples were only collected from patients with a positive BM2 sample.”

**Discretionary Revisions:**
One the main limitation of data presented is absence of correlation with clinical outcome. Last patient was accrued to the SATT study in May 2008, therefore it seems, that the data might be mature for assessment relapse-free survival. Without this information, it is hard to discuss which of utilized method and/or biomarker is more appropriate for DTC detection. If these data are available, or could be collected in timely manner, they should be presented.

*We agree with referee #1 that the data are mature for assessment of relapse-free survival. Without this information it is hard to make final conclusions regarding which of the utilized methods and/or biomarkers is the more appropriate for DTC detection. However, the survival data for the full SATT study has not yet been published, and we find it appropriate and scientifically correct to wait with the publication of survival data from the current substudy until the survival data for the entire SATT cohort have been presented. This would also allow us to discuss the survival data for the ICC method in relation to the larger cohort, which will provide better statistical test power. Unfortunately, this would not be possible in a timely manner.*

**Referee #2:**
This paper by the Oslo group is about the comparison of two DTC detection methods in a large cohort of early breast cancer patients. This paper is scientifically sound, and no major change has to be done before publication.

**Minor Essential Revisions**
1/ In the manuscript I had to review, the references did not appear in the text. The reference list looks good, yet I was not able to check each reference was quoted at the right position.
Unfortunately the references disappeared almost immediately prior to submission for some technical reason, but this is now corrected. We are sorry that this slipped in the first place, and grateful that the paper has been considered without the referenced inserted.

2/ 3 typos should be corrected in Table 1, as the total number of patient (in the 2 column) does not match the sum of ICC Pos + ICC Neg (4th and 5th column) for patients <55 yo , patients 55-70 yo and pT1c tumors. The numbers have been corrected.

Background 3/ 5th line : "has" instead of "have" 4/ 10th line : "does not guarantee disease relapse" is not a scientific wording.

“Have” has been corrected to “has”. We agree that the phrase “does not guarantee disease relapse” is not a good scientific wording and it has been revised accordingly:

“Furthermore, the detection of tumor cells in the BM does not always lead to disease relapse. Many patients with positive DTC status do not relapse, and DTCs can be detected in patients with ductal carcinoma in situ.”

- Discretionary Revisions
5/ In the discussion, I would remind the reader the superiority of the CellSearch assay over the Adnatest (a report by German groups) - which also developed TWIST1 RT-PCR.

We are aware of the reports where CellSearch is suggested to be superior to the Adnatest. However, these studies are to our knowledge performed on blood samples and thereby refer to CTC detection and not DTC detection which is the focus of our study. We believe a more detailed discussion regarding this interesting report might confuse the reader and would therefore suggest that we do not incorporate this into the current manuscript.

I would also mention illegitimate transcripts in activated white cells as a source of noise for RT-qPCR techniques.

We have added the following paragraph to the manuscript:

“A general issue regarding mRNA-based DTC detection is the background level of epithelial transcripts in white blood cells. However, comparison with blood samples from a normal control cohort may compensate for this issue, allowing threshold values for pathological marker levels in blood to be established. The latter strategy was utilized in the current study to minimize the number of false positives due to such background expression in leukocytes.”