Author's response to reviews

Title: The presence of tumor associated macrophages in tumor stroma as a prognostic marker for breast cancer patients

Authors:

Catharina Medrek (catharina.medrek@med.lu.se)
Fredrik Pontén (fredrik.ponten@igp.uu.se)
Karin Jirström (karin.jirstrom@med.lu.se)
Karin Leandersson (karin.leandersson@med.lu.se)

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

We thank you for the opportunity to submit this revised form of our manuscript. We have now answered all the referee comments point by point and also changed the manuscript accordingly (attached as Revision letter). We have also made linguistic corrections to the manuscript. With this letter I kindly ask you to consider the manuscript as suitable for publication in BMC Cancer after this revision.

Yours Sincerely

Catharina Medrek
MD, PhD student, Center for Molecular Pathology, Lund University, Sweden
Reviewer #1: 
Reviewer's report Title: The presence of tumor associated macrophages in tumor stroma as a prognostic marker for breast cancer patients 
Version: 1 Date: 15 March 2012 
Reviewer: carolien van deurzen Reviewer's report: 
The authors report on the prognostic value of tumor associated macrophages using different immunohistochemical markers and conclude that both presence and localization of tumor associated macrophages (TAM) have a prognostic value. The manuscript is well written; I have one major and several minor suggestions.

Major:
The authors report on TAM in tumor stroma and tumor nest and conclude that TAM in tumor stroma is clinically relevant. However, according to figure 1, TAM seem to be located between tumor nests in all cases. The only difference seems the amount of stroma between the tumor nests: there is less stroma in figure B and D. With other words: the division in TAM in tumor stroma versus tumor nests seems to be related to the amount of stroma between the nests. Previous studies suggested that stroma rich tumors have a worse prognosis compared to stroma poor. So, how do you know that you are looking at the prognostic value of TAM: could it not be the amount of stroma mimicking a TAM effect (or it could be related)? This item could be discussed in the discussion part of the paper.

We appreciate this comment very much and therefore we have tried to evaluate the amount of stroma in our cores on the TMA. We are however, at the moment unable to quantify this in an appropriate and satisfactory way. We have therefore added a section to the discussion about this to highlight the possibility (see page 10).


We have now changed this to Ki67 positivity.


Thank you for sharing this new article – we have added it in the reference list!

3. Methods: clarify the definition of Luminal A, B, etc. Is it based on immunohistochemistry?

We have now added this in the methods on page 12 and hope that it is clearer.

4. Methods: Is it true that 79% are luminal A or do you mean luminal A or B?
Yes it is true that 79% are luminal A.

5. Results/methods: Add information regarding the following stainings: anti-DC-Lamp, Granulin, CD208. Provide some background information what these markers stain and why they are used (could also be added in introduction)

DC-LAMP/CD208 is a marker for mature myeloid DCs that also might stain positive for CD163. Granulin is a marker that stains instigating tumor-resident bone marrow derived cells that previously was shown to correlate to TN breast cancers (see discussion page 10). We have added this information in the methods section (page 13) and also changed DC-LAMP to CD208 in a more consistent manner.

6. Methods: How are Ki67, ER, PR scored? What is defined positive?

We have now added this information in the methods section.

7. Results: (fig 1F): What about the gene expression profile of CD68 and subtype?

The CD68 expression levels were also increased in basal like breast cancer although not as much. We have added this information as statistical values in the text on page 7 now.

8. Results: page 8: reference to FIG2E and F seems inappropriate.

Thank you for noticing this. We have now removed “Fig 2E and F” from the text.

9. Discussion: page 10: CD163+ macrophages could represent GRN+ cells: this could be confirmed by double staining, although not necessary for this study.

We agree on this comment. We hope to be able to elaborate on this particular issue in future studies and therefore kindly ask for permission to leave it out from this manuscript.

10. Discussion: page 10: clarify the abbreviation MDC.

We have spelled out this abbreviation the first time it appears in the text (on page 6) and also added it in the abbreviation list.

11. Discussion: page 11: Luminal A tumors are the ones positive for ER, so these are the patients eligible for endocrine therapy. The triple negatives are the ones with more abundant CD163 if I understood correctly. These patients are unlikely to respond to hormonal therapy based on ER/PR negativity, so I don’t think you directly link endocrine therapy to CD163 expression by suggesting that endocrine therapy might be a disadvantage in patients with
abundant CD163 expression.

We agree to this comment and have therefore removed fig 4 C and D.

12. Figure legends: clarify the references to the subdivisions in the figure (A, B etc). Legend of figure 2 is missing.

We are sorry for this mistake, we have now changed it for each comment.

13. Table 1: explain abbreviation (ie. nhg) 14. Figure 2: A and B could be deleted

We have now clarified the abbreviations in the text to the Tables.

We prefer keeping panels A and B, since they visualize and justify the cutoff chosen for the dichotomized variable used in the following analyses.

15. Figure 4 (according to comment 11): luminal A overlaps with the group treated with endocrine therapy and triple negative breast cancers overlap with the ones not treated with endocrine therapy, so these results are expectable and do not provide additional value.

We agree to this comment and have therefore removed fig 4 C and D.

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 interests
Reviewer #2:
Reviewer's report Title: The presence of tumor associated macrophages in tumor stroma as a prognostic marker for breast cancer patients Version: 1 Date: 2 April 2012
Reviewer: JJ Going Reviewer's report:

- Major Compulsory Revisions <none, but the paper would definitely be better if the authors could condense the discussion particularly into a more condensed form >

We have condensed parts of the discussion and hope that it is clearer now.

- Minor Essential Revisions
  1 Background para 2 line 4 'tune out' informal - suggest 'downregulate'

We thank you for this suggestion, we have now changed it in the text.

  2 Background para 3 line 2 'regarded as a ...'

Again, thank you for noticing this, it is now changed in the text.

  3 Methods para 1 line 6,7: Follow up time - is this for all patients, or survivors only?

Thank you, this information has now been modified (denoted as years to harmonize with the survival plots) and further clarified as follows:

"Median follow-up was 6.55 years (range 0.33-7.55 years) for the full cohort and 6.74 years (range 4.88-7.55) for patients alive"

- Discretionary Revisions / < none >
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