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 tumorstroma 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.

**Minor:**

3. Methods: clarify the definition of Luminal A, B, etc. Is it based on immunohistochemistry?
4. Methods: Is it true that 79% are luminal A or do you mean luminal A or B?
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)
6. Methods: How are Ki67, ER, PR scored? What is defined positive?
7. Results: (fig 1F): What about the gene expression profile of CD68 and subtype?
8. Results: page 8: reference to FIG2E and F seems inappropriate.
9. Discussion: page 10: CD163+ macrophages could represent GRN+ cells: this could be confirmed by double staining, although not necessary for this study.

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

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.

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

13. Table 1: explain abbreviation (ie. nhg)

14. Figure 2: A and B could be deleted

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.

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