Reviewer's report

Title: High STAT1 mRNA levels but not its tyrosine phosphorylation are associated with macrophage infiltration and bad prognosis in breast cancer

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Reviewer: Hazem Ghebeh

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Re: 'High STAT1 mRNA levels but not its tyrosine phosphorylation are associated with macrophage infiltration and bad prognosis in breast cancer'

Piotr Tymoszuk, Pornpimol Charoentong, Hubert Hackl, Rita Spilka, Elisabeth Müller-Holzner, Zlatko Trajanoski, Peter Obrist, Françoise Revillion, Jean-Philippe Peyrat, Heidi Fiegl and Wolfgang Doppler BMC Cancer Research article

Dear Dr Dafne Solera PhD

There is major improvement in the manuscript now. However, important deficiencies still exist and should be considered. The most important and major issue is that authors do not have enough evidence for an association between macrophage infiltration and STAT1 mRNA levels (Title). If both STAT1 mRNA and CD68 are associated with bad prognosis, this does not proof that they are associated with each other (Reply to reviewers, page 2 general comments). Therefore this should not be present in the title as their major finding. The data presented have merely showed an association with immune cell infiltration in general.

Other more specific notes:

1. The second subtitle of the results "No evidence for downregulation/mutation... (Page 9)

Having same level of INF-gamma between tumor and adjacent tissues does not exclude the possibility that increased levels of mRNA STAT1 and IRF-1 are not due to immune cell infiltration. IFN-gamma is not always produced by immune cell infiltration. Some types of T-cell activation lead to the release of other cytokines (very common in cancer) like IL-10, TGF-beta without INF-gamma. Interferon-gamma producing macrophages are not always present among tumor infiltrating immune cells.

2. The third subtitle of the results "Coordinate regulation of STAT1, STAT1 target genes.... (page 9 & 10)

The way genes are categorized looks confusing/not accurate:

a) What is the justification for grouping CXCL9, CXCL10, CXCL11 together? These chemokines can be produced by many cells in addition to epithelial cells and therefore cannot be claimed that they specifically linked with STAT1
expression by tumor epithelium.

b) What is the justification for grouping INF-gamma, CD45, and FOXP3 together? INF-gamma is not a specific lymphocyte marker as it is released by both lymphocytes and macrophages (see review by Schroder et al 2011). CD45 is not a lymphocytes marker, it is rather a pan-leukocyte marker (lymphocytes, neutrophils and monocytes all express CD45). FOXP3 is a marker of a subset of lymphocytes (T-reg) but it can be expressed by tumor cells (see Triulzi et al 2013).

c) PD-L2 is expressed on activated T-cells and not macrophages and dendritic cells only as initially thought (reviewed by Rozali et al 2012).

d) What is the significance of two clusters? What does this finding means? ( I don't see what figure 5 is adding to the main findings of the paper...).

3. Discussion
This part has to be improved as it is the most interesting finding in the paper

a) The significant correlation between STAT1 mRNA and STAT1 protein phosphorylated at Ser727 but not with Y701 should be discussed more as there might be difference in the impact on STAT1 activation by the site of phosphorylation. For example: recent findings by Barnholt et al. 2009 have shown that macrophage activation can be reduced by inhibition of Ser727 phosphorylation but not Y701 indicating a difference in the role of these two sites of phosphorylation.

b) In addition, the mechanism and importance of the activation of STAT1 between different subset of cells in breast cancer should be discussed (see for example Koromilas et al 2013).

c) The fact that some genes are controlled posttranscriptionalaly should be mentioned and caution should be made not to consider mRNA equal to protein expression always. Based on this, statements like "STAT1 mRNA levels as compared to STAT1 protein (IHC) provides an argument against a direct involvement..." (page 13) might not be justified as mRNA does not always mean protein. This is in addition that the type of cell producing this mRNA is not known.

d) Discussion in page 14 need to be improved, abridged (currently it has contradictory or not relevant sentences/paragraphs).

References for the comments:


**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