Author's response to reviews

Title: Prognostic impact of cd57, cd68, m-csf, csf-1r, Ki67 and TGF-beta in Soft Tissue Sarcomas

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Author's response to reviews:

The comments from the reviewers are all valuable and very helpful for improving our paper. We have studied comments carefully and have made corrections.

Reviewer 1: I do note that on page 4, 4th line, there are 2 references - 1 is in the proper format and the other simply has an author name and year. It appears that the reference that is not properly formatted is not in the reference list, though I did find it in pub med.

Our response: The reference (Sørbye 2012) is now updated and properly formatted.

Reviewer 2: Although the writing in general is very good, the whole manuscript should be revised for some minor inconsistencies. For example, on p.3, line 2: “…more than 50 different histological entities and [they] comprise less than...”; p.4, l. 4 the term (Sørbye 2012) # can be deleted, the numerical reference [25] is enough, same in l.17; p.5, l.10: change carcinomasarcomas to carcinosarcomas?; the legend in table 2 and 3 is displaced (Patient-s); in figure 2, last graph: TGF-beta is spelled wrong (TFG-beta); also: personally, I prefer the abbreviation STS also in the plural without an additional “s” (“STSs”, f.e. p.3, l.3 or p.11, l.6).

Our response: All the minor inconsistencies are now corrected. Thank you very
much for your comments on this paper.

Reviewer 2: The finding, that Ki67 in the capsule is also a predictor of prognosis, was only shortly discussed by the authors. Was a correlation to metastasis tested statistically? May someone hypothesize, that an increased expression of Ki67 can be the result of an increased migration of fast-proliferating cells in the peritumoral capsule or could there be an enhanced proliferation effect of tumor-released cytokines on the stromal cells? Maybe, the authors could discuss this finding in a few sentences more.

Our response: That is a good question. There was a correlation of expression of Ki67 in tumor (N=249) and metastasis at the time of the diagnosis (P=0.001), but not a statistical significant correlation of expression of Ki67 in peritumoral capsule (N=80) and metastasis at the time of the diagnosis (P=0.395), maybe due to low numbers and low statistical power. There were only 15 out of 80 patients with tissue from peritumoral capsule who had metastasis at the time of the diagnosis. We have now added a few sentences in the results and in the discussion.

Reviewer 2: In figure 2, univariate analysis of co-expression M-CSF and TGF-beta shows only 12 patients in the low M-CSF, high TGF-beta group. Are there any literature pointing towards a regulation of M-CSF expression by TGF-beta? In the univariate analysis presented TGF-beta seems to be the dominating factor, while low or high M-CSF expression in combination with low TGF-beta expression does not seem to influence prognosis significantly. Maybe, the authors could discuss this impression in one or two sentences.

Our response: That is also a good question. There was a co-variation between expression of M-CSF and TGF-beta in tumor (P<0.001). In crosstabulation the expected count in the low M-CSF, high TGF-beta group was 26.7 patients, but the observed count was 12. Grayfer et al. reported on the regulation of pro-inflammatory functions of goldfish macrophages and induction of gene expression by recombinant goldfish CSF-1 (rgCSF-1). In addition to being an important growth factor of goldfish macrophages, rgCSF-1 also plays a central role in the regulation of their pro-inflammatory responses. At 72h post treatment rgCSF-1 increased the expression of TGFbeta (Grayfer 2009). The combined expression of immunostimulatory granulocyte macrophage colony stimulating factor (GM-CSF) and antitumor suppressor TGF-#2 antisense (AS) transgenes can break tolerance and stimulate immune responses to cancer-associated antigens which make it possible to design bifunctional therapeutic anti-cancer vaccines (Olivares 2011). Both expression of TGF-beta and M-CSF have co-variation with malignancy grade and expression of Ki67. In the multivariate analysis the co-expression of M-CSF and TGF-beta was a stronger prognosticator for DSS than each of the markers alone. We have now added a few sentences in the results and in the discussion. Thank you very much for your comments on this paper.
Reviewer 3: There are no preliminary considerations about the markers used, with exception of Ki-67. This would be important to inform the readers about the reasons why the authors chose these and not other markers of immune response.

Our response: That is a good suggestion. We took reviewer’s advice. We have previously reported the prognostic significance of the humoral immune system by lymphocyte infiltration in tumor (Sørbye 2011) and peritumoral capsule (Sørbye 2012) of STS. We have also reported the significance of the innate immune system by the correlation of expression of macrophages (CD68), their growth factor macrophage colony-stimulating factor (M-CSF), its receptor colony-stimulating factor-1 receptor (CSF-1R) and histological grade in STS (Richardsen 2009). It was important to validate these findings in a different material, explore the relationship to expression of Ki67, disease-specific survival (DSS) and include other markers as CD57 and TGF-beta.

Reviewer 3: In the last paragraph, the authors refer to an article by Sorbye 2012 and to the reference #25 (2011). Are there two articles, or are they the same. If this is the case, only the number is sufficient.

Our response: There are two articles, but Sørbye 2012 was not published at the time this manuscript was submitted. Now both articles are published and the references are updated.

Reviewer 3: Methods: Some details could have been summarized, in order to condense this section.

Our response: We agree. We have cut down some sentences and referred to our previous article for more details.

Reviewer 3: On the other hand, abbreviations like “CK, SMA, VIM” should be defined when used for the first time: cytokeratin, smooth muscle actin, vimentin, respectively.

Our response: We agree. The definitions are now included.

Reviewer 3: Again, in the second paragraph the authors refer to an article by Sorbye 2012. Please, clarify (reference #25? Other not cited in the list of references?)

Our response: The reference (Sørbye 2012) is now updated and properly formatted.

Reviewer 3: In the subsection “Immunohistochemistry” the authors refer to CD56,
and not to CD57. Thereafter, scoring, results and discussion is made on CD57. Please, clarify.

Our response: Thanks for reviewer's careful review. We have used the immunomarker CD57. The subsection is now corrected.

Reviewer 3: While two large paragraphs are dedicated to the role of CD57 in tumor immunity, few is commented on the most significant markers, M-CSF and TGF-beta. This should be readdressed, especially because the authors made a so strong statement in the last paragraph “This data may provide additional information to guide therapy after surgical resection”.

Our response: We agree. We have now included more about the role of M-CSF and TGF-beta. Thank you very much for your comments on this paper.