Reviewer’s report

**Title:** S100A4-neutralizing antibody suppresses spontaneous tumor progression, pre-metastatic niche formation and alters the T-cell polarization balance

**Version:** 3 **Date:** 10 November 2014

**Reviewer:** Gunhild Mari M Mælandsmo

Reviewer’s report:

Comments from reviewer

In the study by Grum-Schwensen et al in vivo models are utilized to demonstrate that the pro-metastatic protein S100A4 shift the T-cell population towards the Th2 pro-tumorigenic phenotype and that the 6B12 antibody inhibit metastasis by suppressing the attraction of T-cells to the primary tumor and also to the pre-metastatic lungs. The paper discuss the interesting possibility of utilizing the antibody for therapeutic purposes for instance in combination with cytotoxic therapy. This is an intriguing possibility, but it needs to be evaluated in a variety of models systems utilizing human tumor models before clinical translation can be a reality.

The manuscript suffers from some inaccuracies in the linguistics and a thorough review of the written text should be performed. The strength of the manuscript is that the authors use in vivo models to show the effect of a monoclonal antibody that blocks the metastases-promoting effects of the S100A4 protein. This ability has however been demonstrated by the same group before (Klingelhofer et al, Neoplasia, 2012, 14, -1260-1268) utilizing a slightly different syngeneic in vivo model of spontaneous lung metastasis. For studies of S100A4 in pre-metastatic niche formation, the reviewer miss systematic use of S100A4 +/+ as well as S100A4 +/- MEFs throughout all figures/panels and how these control fibroblasts impact on the different parameters when adding both the S100A4 blocking antibody and also the control IgG.

Major Revisions:

The in vivo data showing reduced spontaneous tumor development and metastasis formation (Figure 3) is convincing, but representative pictures showing vessel density and T-lymphocyte infiltration should be presented.

In the pre-metastatic niche model (Figure 4) an essential control will be to quantify the impact of control fibroblasts (S100A4-/- MEFs), measured as fibronectin expression (Panel B) and T-cell infiltration (Panel E) and also to use these fibroblasts as control in all experiments evaluating the impact of the S100A4 blocking antibody and control IgG. This is of particular importance since the effect of S100A4+/+ MEFs is rather modest. It is also unclear whether the results presented in panel C and D is from animals injected with S100A4+/+ MEFs or only tumor cells.
Minor Revisions:

In Table 1, lungs from five animals are harvested, incubated in PBS and pooled. Statistics showing the variation between animals in each individual group, and whether the difference between animals injected with S100A4-/- vs S100A4+/+ MEFs should be given since several of the mentioned cytokines are responding in both groups (eotaxin-2, IL9, IL4IFN-gamma). According to the table, several other cytokines are also responding to the same degree without being mentioned in the text (e.g., Lix, SDF-1alpha, PF4, ctack, CXCL16). How could this be interpreted?

Few details regarding how experiments have been performed, and no statistics in the presented data are given in supplementary Table 1 and 2. From where have the lymphocytes been harvested? From control animals or animals given S100A4-/- or S100A4+/+ MEFs? How many mice have been analyzed? What are the variations between the animals? How many parallels have been analyzed?

Some inaccuracy is also present in Figure 2. How many experiments have been performed. Only three animals seem included in panel E and with borderline statistical significance this could have been repeated with more animals in each group. Incubation with a control IgG antibody is also lacking in the panels where 6B12 have been used.

Level of interest: An article of importance in its field

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