Reviewer's report

Title: EphB4 as a therapeutic target in mesothelioma

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Reviewer: Sally Stephenson

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In this study the authors were interested in determining the effects of a soluble form of the EphB4 receptor extracellular domain on in vivo growth of EphB4-positive tumors. This is a question very familiar to this group, having already published studies reporting EphB4 over-expression in mesothelioma (Xia et al, Clin Cancer Res 2005) and testing the effect of sEphB4 and sEphB4-HSA proteins on tumor growth in vivo using cell lines representing several cancers (Kertesz et al, Blood 2006). For these reasons this study is not particularly novel but rather supportive of work they have already published.

In the first result of the current paper, Liu et al confirm their previous result showing EphB4 is commonly over-expressed in mesotheliomas. They then choose a model cell line (NCI-H2373) to test the effect of sEphB4-HSA proteins to inhibit the growth of established xenograft tumours. Analysis of the tumour tissue suggested that there was less tumor angiogenesis, tumour cell proliferation and more apoptosis in the animals treated with sEphB4-HSA. Combined treatment of tumors formed using a second cell line model 211H with sEphB4-HSA and the anti-VEGF-A antibody Bevacizumab appears particularly effective. This is also consistent with their previous studies.

Minor Essential Revisions

1. The authors should explain their choice of H2373 and 211H as the model cell lines for different parts of the study.

2. The authors describe the loss of pAkt, pS6 and pSrc as “persistent through the length of the study (25 days)” but they cannot really say this given analysis was only performed after 25 days of treatment. This should be reworded.

3. In Figure 4, the co-localisation of CD31 and NG2 is obscured by the addition of the DAPI staining. Can the authors include a panel without the DAPI?

4. Please include images of the positive control cells used in the immunohistochemistry experiments – 293T expressing EphB4, with a comparison to the parental 293T cells as validation of the specificity of the antibody staining.

Discretionary Revisions

1. The data showing loss of pAkt, pS6 and pSrc could have been supported by in
vitro studies.

2. ephrin-B2 signaling is also mediated through PI3K and Src and sEphB4-HSA may be preventing ephrin-B2 activation. Confirmation that the loss of immunoreactivity seen in the tissue was from the tumour cells and not the supporting cells (endothelial cells and pericytes) would be valuable.

Several typographical errors were noticed including

1. Page 3 Backgraound should be Background
2. Page 3 kinase kinase should be kinase
3. Page 4 BD biosciences should be BD Biosciences
4. Page 6 phsophrylated should be phosphorylated
5. Page 12 Bevacixumabon should be Bevacizumab on

**Level of interest:** An article of importance in its field

**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