Reviewer’s report

Title: High expression of EphA3 (erythropoietin-producing hepatocellular A3) in gastric cancer is associated with metastasis and poor survival.

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Reviewer: Chris Slape

Reviewer's report:

The manuscript by Nasri et al measures the expression of EphA3 in gastric cancer specimens by IHC and then uses statistical analysis to associate expression with clinical features.

The authors' findings suggest that EphA3 is not an independent prognostic factor for gastric cancer, but is associated with worse outcome.

The results are of interest although not entirely novel as EphA3 expression in gastric cancer has already been reported to correlate with poor prognosis and survival (Xi et al 2012).

Major compulsory revisions:

There are concerns over the specificity of the antibody used - Santa Cruz lists human kidney as a positive control for this antibody, yet EphA3 is not expressed in adult human kidney. Santa Cruz is withdrawing this product from sale and the "replacement" antibody is not recommended for use for IHC. Positive and negative controls for EphA3 are essential to demonstrate that this antibody is reactive and specific for EphA3.

Authors state that the normal gastric epithelium samples were negative for EphA3 staining however there is immunoreactivity in Figure 1A. Can authors please include images of their IgG (Isotype control) or no primary antibody controls to clear up confusion as to whether there is positive staining in the normal samples.

Scale bars should be included on all images.

Minor essential revisions:

Multiple passages are directly lifted from other research articles - while every instance of this that we identified was correctly referenced, it is inappropriate to copy whole sentences word-for-word.
Although I am not qualified to review the application of statistics in this article, the statistical analysis techniques appear to be consistent with standards for this type of study. A little more clarity in the Methods section regarding this might be helpful, and the sentence "Prognosis was evaluated based on the length of Overall Survival and Relapse Free Survival" should be edited for clarity.

Table 1 has categories for "distant metastasis or recurrence", etc, when patients with distant metastasis were specifically excluded from the study and so any such metastasis that occurs later should not be recurrent. More clarity surrounding inclusion/exclusion criteria would be beneficial, as would clarification of the categories in the table.

Authors state that staining extensity was classified into four categories, but then go on to list only three categories. (I imagine the authors originally had four categories where the lowest was "0%" rather than "0-9%". In my opinion 0% should be considered in its own category, but this is discretionary.)

When authors state "separately counted stained tumor cells in at least three field per section, including the deepest site invaded by tumour cells, the surface of the lesion and the intermediate zone" does this mean three fields per section, or one field per section for three sections?

Discretionary revisions/comments:

I am uncertain of the value of combining "staining intensity" and "staining extensity" into a single composite score, given that they reflect very different biological situations. Why not analyse each component separately? However this is not an uncommon practice and so need not be corrected for publication.

It's not clear from the introduction why EphA3 was chosen to study.

The Discussion is largely irrelevant to the Results. There is substantial discussion of mechanism which is wholly unaddressed by the analyses performed in this study and this section could be greatly reduced in length. Discussion should also mention the limitations of the study, and discuss in more depth the differences between their study and the recently published Xi et al study.

Statistical review:

I do not feel adequately qualified to assess the statistics

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.
No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

No

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I recommend additional statistical review

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Needs some language corrections before being published

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