Dear Editor,

RE: MS: 3025267859288564 - Over-expression of Eph and ephrin genes in advanced ovarian cancer: ephrin gene expression correlates with shortened survival  
Authors: NI Herath, MD Spanevello, S Sabesan, T Newton, M Cummings, S Duffy, D Lincoln, G Boyle, P Parsons, AW Boyd

Thank you for the reviewers and your comments regarding the above manuscript. We have addressed the issues raised point by point and have enclosed a revised version of the manuscript and author contributions.

If you have any questions please do not hesitate to contact us.

Yours faithfully,
Nirmitha Herath

Editor's comments

1. Please comment on the sample size used (re reviewer 1), what is the power of this study?

This has also been addressed (see Referee 1, major revision)  
The statisticians have advised us that power calculations are not informative for the present study as the
results reached statistical significance with this sample size. This may be more relevant in the proposed large, prospective analysis, particularly if no statistical significance is shown but trends are identified. Relevant references provided by the Statistics Unit:
b) The overemphasis on power analysis. Thomas Knapp. Nursing Research 1996: 45(6); 379

2. Please cite previous publications regarding the Eph receptor in ovarian cancer.

This has been included in the revised discussion

3. If possible please record what percentage was tumor in the tissue samples?

We did carry out a careful analysis of the tumor sections, including % tumor and other factors such as evidence of EMT transition in the tumors. This was not presented as there was no obvious trend, and in addition, we believe the sections constituted only a small fraction of the tumors and might not be representative. We have added the sentence "Survival also did not correlate with pathological categorization of tumor grade or cellularity." to the Results section.

Referee 1

Major revision

1. The study is limited by the small number of samples examined.

Whilst we acknowledge that the sample size is relatively small, the highly significant correlations in this analysis prompted us to publish these findings (please see response to editor’s comment 1). The last sentence of the discussion has been altered to emphasise the limitations of the report.

"Whilst this study has yielded surprisingly strong correlations, a prospective study of a much larger cohort is warranted to further assess the use of ephrin expression as a useful predictor of clinical outcomes in this disease."

2. Reliance on one modality is a limitation. Correlation with protein expression (IHC/western) in these specimens would add strength to the submission, at the least as supportive data in the specimens in which correlation is found on RTPCR

We used one in house and three commercial antibodies to EphA1 in immunohistochemical studies of paraffin embedded tissues from these patients. None of these antibodies were satisfactory due to very high background and non-specificity in these tissues. We trialled three antibodies which were claimed to work on mouse and human cells to stain wild type and EphA1 knockout mouse tissues. The staining was equivalent for both, confirming the lack of specificity of these antibodies. The failure to make good antibodies may reflect the high degree of interspecies identity of these proteins. In fact, we are currently developing monoclonal antibodies against EphA1 and EphA2 in KO mice in an attempt to overcome these problems. These may be useful for future studies but are not yet available.

However, to address this point we have conducted a study of the ovarian cancer cell lines, OVCAR4 and OVCAR5, to compare quantitative real-time PCR and Western blotting analysis. In each case EphA1 expression was significantly higher than EphA2 which was confirmed through both real time PCR and Western blotting. We have added a sentence in the discussion in part to answer the reviewer’s query.

3. The mechanistic conclusions are not supported by data in this submission. Have the authors examined other components of Ephs and ephrin signalling?

We have amended the discussion to indicate that these comments are speculation rather than strong conclusions from the presented data.

Minor revision

1. The reviewer asked for the differences between the current study and that of Han et al 2005 to be discussed. Han et al demonstrated that over-expression of EphA2 was associated with shorter survival in patients with ovarian carcinoma. Since ephrin A1 and ephrin A5 are high affinity ligands for EphA2, the findings from the current study complement these data. This has been included in the discussion.
2. Moderate correlation has been amended to "trend"

Referee 2
Minor revision

1. Correlation of ephrin A5 with poor survival has been corrected to p<0.01 in Abstract: Results

2. Specific Ephs and ephrins added to the Abstract: Conclusions

3. "Gene copy number" has been amended to "Transcript Number" in Figure 1 and in Figure 1 legend.