Reviewer's report

Title: Elevated cyclin B2 expression in invasive breast carcinoma is associated with unfavorable clinical outcome

Version: 1 Date: 2 October 2012

Reviewer: Rebecca Lamb

Reviewer's report:

Major Compulsory Revisions
1) It would be useful to examine all protein expression in relation to clinicopathological features without the inclusion of DSS as a first stage of analysis.

2) Figure 2: It would be useful to explain in the figure legend what is different between A, B, C, D, . Is this trying to show varying expression levels, if so explain this.

3) Figure 2: The image in figure 2A appears to be a different magnification, if this is correct please state this in the legend.

4) Table 2: There are two p values indicated within the table, both determined from a chi-squared test. It needs to be much clearer what these p values are assessing.

5) Within the text on page 11 when referring to figure 4, you state “mRNA levels for Her2/neu (p value). However I can’t see any reference to Her2 mRNA expression within this figure, or any other figure.

6) You have used mRNA expression to validate the protein expression observed using IHC. Where they correlate you assume the antibody is working correctly, and when they do not correlate you assume that this is due to posttranslational modifications. Although it is good that you have attempted to validate your antibodies, this is perhaps not the best method. It may be that the reason for the discordance in mRNA and protein is that the antibody for IHC is working poorly in comparison to the primers used to detect mRNA expression. This should at least be considered as a limitation.

Minor Essential Revisions
1) Figure1: It would be useful to put CCNB2 into the figure itself, ie CCNB2 expressed/CCNB2 not expressed.

2) Table 2: State in legend (n=number (%)) as in table 1.

3) Table 2: There numbers are missing within the table for CCNB2 expression.

4) p values, and CIs have been displayed incorrectly. Ie p value states 0.0 this should be 0.003 etc.
Discretionary Revisions

1) It would be useful to describe (show images) if CCNB2 is expressed in normal breast tissue.

2) It would be good to see examples of the IHC for the other genes tested to enable future researchers to compare their staining.

3) Within the discussion on page 13, you talk about the differences in mRNA and protein expression within the tumour perhaps being accounted for by the inclusion of all cell types within the tumour for mRNA expression. Perhaps you could look at the IHC staining within tumours to identify whether other cell types within the tumour also express the proteins.

4) Is there any evidence, or do you have a hypothesis regarding the nature of the interaction of CCNB2 and ASPM. For example, is this a direct functional interaction between the two genes, or just that both are independently activated?

5) To properly validate the IHC antibody a more appropriate method would be to either overexpress /inhibit protein expression and confirm this using the IHC antibody. Or to validate the antibody using tissue/cell lines of known and varying CCNB2 expression.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests