Author's response to reviews

Title: HP1-gamma expression is elevated in prostate cancer and is superior to Gleason score as a predictor of biochemical recurrence after radical prostatectomy

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Author's response to reviews: see over
December 20, 2012

Dear Editor,

Thank you for your consideration of our manuscript. We have included detailed responses and manuscript revisions, addressing each of the reviewer’s points.

Sincerely,
David Jarrard

Reviewer: Susan K Logan

Reviewer’s report:

This manuscript examines expression of the heterochromatin protein HP1 gamma and Ki67 in prostate cancer.

Minor essential revisions:

On page 8 the authors indicate that HP1 gamma is over-expressed in 26% of benign cores. Please explain how this marker has predictive value in light of this fact.

**Author response:** There is a distinct difference between the proportion of patients with high versus low Hp1 gamma expression using cut-off values for mean intensity. While this cut-off was determined by ROC analysis to highlight the marked differences between patients with benign versus cancer, adjusting the cut-off for Hp1-gamma overexpression can increase the sensitivity or specificity depending on the intended purpose. A statement was added to the discussion to address this.

Page 9, top. The text sounds as if the correlation were strongest for HP1 gamma and Ki67 cytoplasmic expression. Since Ki67 is nuclear this is hard to interpret. However, the figure indicates that it is always nuclear Ki67 being correlated with total, nuclear or cytoplasmic HP1 gamma expression. Please clarify in the text.

**Author response:** This was performed on a per-core basis, not a per-cell basis. The phrase “on a per core basis” was added to the discussion of the correlation analysis for clarification. The correlation was significant between nuclear Ki67 and all compartments of HP1gamma. The unknown significance of cytoplasmic HPIgamma was addressed in the discussion.

Page 12. Please clarify the sentence “One explanation for the robustness of HP1 is that its prognostic value is not confounded by other factors ....".
**Author response:** We clarified this sentence in the discussion. Hp1-gamma is not collinear with other features of poor prognosis.

Figure 1 is difficult to interpret. For example, figure 2 shows only a 1.5-2 fold difference in HP1 gamma staining between benign and cancer specimens, but Figure 1 shows no HP1 gamma staining. Is the staining really all or none? It is not clear if the benign and cancer samples shown here are at the same magnification. For clarity, it would be helpful to have the H & E staining if it is possible.

**Author response:** Figure 1 was modified to include additional images from benign and low grade cancer. Staining is not all or none rather there is always some expression in benign in virtually all cells. VECTRA is quantitative and extremely sensitive. Figure legend was clarified state that Hp1 gamma is expressed in virtually all benign cells to a low degree. We believe these additional images are clearer and do not require the correlating H&E.

Reviewer: William B Isaacs

Reviewer's report:

Major revisions

1. Unless appropriate references can be cited, the antibody used needs to be validated by western blot and appropriate positive or negative controls included.

**Author response:** We have added a reference for the Hp1-gamma antibody.

2. Additional images should be provided - the single image of cancer provided is almost too good to be true!

**Author response:** We have provided additional images for benign tissues and also provided 2 cancer images, one with Gleason 3+3 and another with Gleason 4+4. Hp1-gamma overexpression is very robust and uniform in cancer.