Reviewer’s report

**Title:** Urinary Levels of Hepatocarcinoma-intestine-pancreas/Pancreatitisa-associated Protein as a Diagnostic Biomarker in Patients with Urothelial Carcinoma

**Version:** 1  **Date:** 6 June 2012

**Reviewer:** Charles Rosser

Reviewer’s report:

This is an interesting study by Nitta et al. describing HIP/PAP in ‘tissue’ and urinary of subjects with bladder cancer. A couple of publications (Orenes-Pinero and Geng) previously reported the potential utility of HIP/PAP as a bladder cancer tumor marker. In this report, the authors show elevated urinary levels in subjects with bladder cancer and then hint that HIP/PAP may be a prognostic factor as well. Overall this is an intriguing paper, however attention must be given to grammar and formatting. Issues related to grammar and formatting distract from the message of the article. Below is a point by point assessment of the manuscript.

**TITLE:** No issues

**ABSTRACT:** The methods section requires more details as to patient numbers, patient conditions, cell lines, etc. The word superficial is used throughout the manuscript. The correct terminology would be non-muscle invasive bladder cancer. Should stress the preliminary nature of these results.

**BACKGROUND:** The first sentence “Bladder cancer is the most common urothelial carcinoma is confusing. I think you mean to say Urothelial carcinoma (formerly transitional cell carcinoma) is the most common bladder cancer. Next statistics from 2008 are given. Can you provide statistics from 2011 or 2012? Previously the authors demonstrated HIP/PAP is increased in inflammatory conditions of the bladder. Does this distract from HIP/PAP as a diagnostic marker since inflammatory conditions will be positive? Perhaps patients with standard inflammatory conditions (cytistis) should be added as a separate control group.

**METHODS:** In the text state the break down of the benign controls. The healthy volunteers may offer little information since their numbers are so low. Subjects with bladder and upper tract tumors are included. Though these are UC, the molecular make-up are different. Please delete subjects with upper tract tumors and only report subjects with bladder cancer. Western blot was performed to illustrate HIP/PAP expression. I think this is ok for the cell lines, but for the human specimens immunohistochemical staining should be employed to confirm its presence and to determine the location of the HIP/PAP. Furthermore more than 1 tumor should be analyzed. Were staff running assays blinded to disease status? How did urinary hemoglobin levels affect assay? As you know urinary
blood can adversely affect NMP-22 and BTA. Were HIP/PAP levels normalized to urinary protein or urinary creatinine? Urinary levels/concentrations may vary widely throughout the day due to hydration status, voiding patterns, etc. Urinary supernatants were frozen and analyzed. Is it possible to reliably measure NMP-22 from previously frozen samples? NMP-22 manufacturers say it's not reliable. BTA is ok. Please consider reporting by STARD and REMARK criteria for biomarkers.

RESULTS: Was there a positive correlation with HIP/PAP with grade as there was with stage? How about tumor size? Please include the levels of HIP/PAP as it relates to low-risk and intermediate risk as well as low progression, intermediate and high progression. Only sensitivity of cytology is reported. This is a limitation.

DISCUSSION: Please include a paragraph on limitations of your study. Is it possible to compare and contrast your data to that of Geng et al.?

CONCLUSIONS: Please stress these results are preliminary.

REFERENCES: No issues

FIGURE 1: Delete normal ureter and ureteral tumor. Add normal bladder (perhaps you may obtain from a patient undergoing prostatectomy. Better yet, IHC should be performed to demonstrate staining and location of staining.

FIGURE 2: Nice figure but does not add much. Please convert to works and add to Methods section stating ELISA is specific for HIP/PAP (data not shown).

FIGURE 3: Please split the control group to healthy volunteers and benign conditions. Median bars are undetectable. Please make more pronounced. Please show graph as to how grade corresponds to progression.

FIGURE 4: Please add high recurrence to (A). Most Figures could benefit from being re-labeled for clarity. This goes in line with grammar and formatting mentioned above.

FIGURE 5: Can BTA be included?

TABLE 1

Please compare numbers, sex, age to controls and tumors.

G1, G2 and G3 are now reported as low grade and high grade

What is LOH? Please write out. What is NGB? Please write out.

Please perform multivariate analysis? Controlling for various factors was HIP/PAP the best diagnostic marker? Controlling for various factors was HIP/PAP the best prognostic factor?

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Not suitable for publication unless extensively edited

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