Reviewer's report

Title: Synuclein Gamma Predicts Poor Clinical Outcome in Colon Cancer with Normal Levels of Carcinoembryonic Antigen

Version: 2 Date: 27 August 2009

Reviewer: David Horst

Reviewer's report:

The study by Liu et al investigates the usefulness of Synuclein expression as a prognostic marker in colon cancer. The observation that SNCG may be of prognostic relevance is in itself interesting. Unfortunately however, all the comparative evaluations about which marker may be better than the other are statistically incorrect and large parts of the manuscript are therefore based on wrong assumptions. As the rationale to include CEA-serum levels into this investigation remains unclear because this is rather a follow-up marker than a prognostic marker, I think the authors would be better off presenting the relevance of SNCG only and getting rid of CEA and the flawed comparative analyses.

Major Points:

1. Introduction: In colon cancer, CEA is mainly used as a marker for serial follow-up to detect tumor recurrence and progression rather than as a prognostic/predictive marker. The rationale to study synuclein as a prognostic marker in conjunction with serum CEA levels is therefore not clear.

2. Materials and Methods: Overall survival should be calculated from the date of surgery to the date of recorded death. Data of patients with lost follow-up should be censored. Same applies for disease free survival.

3. In the results section, where the correlation between SNCG expression and survival is presented, the second half of the first paragraph “A significant difference in survival rate was observed…” is redundant to the first half of this paragraph. Additionally, in the first half a p-value of <0.0001 is presented for survival association, while in the second half a p-value of 0.001 is presented. Which one is correct? Please clarify and remove repetitions.

4. Results: The conclusion that SNCG is a stronger predictive factor than intravascular embolus or weaker than depth of tumor invasion cannot be drawn from the data presented. This conclusion could only be drawn if the 95% confidence intervals of the hazard ratios for both markers were not overlapping.

5. The same applies for the comparison of hazard ratios. If the 95% confidence intervals overlap (which is the case for all data presented in the next paragraph regarding CEA/SNCG combo versus CEA and SNCG alone), no statement regarding which one of the investigated markers is better than the other can be made. Instead they are then assumed to be of NO significant difference in this study population. Therefore all the statements about the suggested superiority of
the marker combination compared to the single marker analyses may be wrong.

6. The same mistake is unfortunately carried on in the next (last) paragraph of the results as well: You really cannot compare pure hazard ratios. For all the markers (SNCG, Tumor size, Tumor differentiation) the 95% confidence intervals are largely overlapping (Table 4). There is NO significant difference in the presented study population for these markers.

7. What is the rationale to look at the subgroup of CEA negative patients in specific? The sample size becomes pretty small and CEA is not a strong predictive marker for colon cancer anyway, rather than a serial follow-up marker. As the information gathered from this subgroup does not add any useful data to the observations among the whole study population, I would suggest to remove this sub-evaluation.

8. Discussion: The discussion is mainly based on the assumed higher prognostic value of SNCG or the CEA/SNCG combo which is unfortunately statistically wrong as it cannot be assessed within the available data. It should therefore be rewritten accordingly.

Minor Points:

9. Mat/Met: Was the analysis carried out on whole tissue sections, or were the cases arrayed in a tissue-micro-array? Please specify.

10. I don’t understand the scoring system for synuclein expression. Specifically “a total score for area adding grade of 3…” remains unclear. Finally the authors only derive a positive and negative category for their statistical evaluation. What is the reason to use the complex scoring system? Please clarify.

11. Results: What exactly was the heterogeneous expression pattern of synuclein? Was there a predominance of staining e.g. at the tumor margins? Or was the staining randomly dispersed among the tumor cells?

12. If 4 cases showed staining of normal mucosa, synuclein is not “specifically” expressed in tumors and is not limited to adenocarcinomas. Please rephrase.

**Level of interest:** An article of limited interest

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests