Reviewer's report

Title: Bax Expression Measured by AQUAnalysis is an Independent Prognostic Marker in Oral Squamous Cell Carcinoma

Version: 4 Date: 13 June 2012

Reviewer: Danielle Camisasca

Reviewer's report:

- Major Compulsory Revisions
  
  - Methods
    1- page 7 (TMA): Clarify in the manuscript text why pre-treatment FFPE were used. The sample was represented then by biopsy samples? If so, make it clear in the text. Hematoxylin and eosin revision and tumor grading was based in these pre-treatment FFPE slides? Once all patients were submitted to primary surgery, the pathology report should be based on surgical specimens, which better demonstrate tumor biology and probable behavior.
    2 - page 7 (TMA): Authors state that “blocks of sufficient quality” were selected. Details about this selection should be given – was the front of invasion used in all cases? How many areas were used to represent each case? What were the criteria used in this selection?
    3 - page 7 (TMA): What was the source of the normal epithelia (OCSE)? Explain it in the manuscript text.
    4 - page 8 Statistical Analysis: Detail Cox analysis (what was the method employed – Wald/Enter? Different models were used? Why was the one presented in the manuscript chosen?). If univariate analysis (Kaplan Meier) was performed to investigate survival probabilities according to clinicopathologic variables, this should be shown in a table and significant variables could also be added to the Cox model.

- Results
  5 - page 10: Ki67 is used as a reference to be compared to Bax, Bcl-2 and Bcl-X expression. As such, it should be included in the Methods section.
  6 - page 11: one or two tables should be added to show the data that are described as “data not shown”.

- Discussion
  7 - page 12: Change the sentence “lack of reproducibility in these studies has precluded their clinical use”. In order to be accepted and published, methods in every research should be able to be reproduced. Even semi-quantitative methods are supposed to be reproducible. At least one study [22] also employs an automated quantitative IHC, which is further explained by Bernardo V et al. in Microsc Microanal. 2009 Aug;15(4):353-65.
- Figure Legends
8- Figure 1: What happened to the 5 or 6 cases that were not included for IHC analysis? Did they fall off the slides? The same 63 patients were analyzed for all three proteins?

- Minor Essential Revisions
9 - Background, page 5, last paragraph: … are expected TO act
10- Results, page 9 – standardize Bax writing (BAX/Bax)

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

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

Non-financial competing interests in relation to this paper:
An article of mine is cited in the references of the present text (Camisasca et al., 2009).