**Author’s response to reviews**

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

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**Author’s response to reviews:** see over
April 25, 2012

Dr. Christna Chap
Executive Editor, BMC Cancer
BMC Cancer
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236 Gray's Inn Road
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Dear Dr. Chap,

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

On behalf my co-authors and myself, please consider this manuscript entitled “Bax Expression Measured by AQUAnalysis® is an Independent Prognostic Marker in Oral Squamous Cell Carcinoma” for publication in BMC Cancer. We believe that this manuscript is of intrinsic relevance to cancer and, consequently, it is appropriate that it should be submitted for publication in a general and open access cancer research journal.

Resistance to apoptosis is a hallmark of cancer; however, the prognostic impact of apoptotic markers has not been consistently demonstrated in oral squamous cell carcinoma (OSCC). We believe that the inconsistency regarding the association of apoptotic proteins with prognosis in OSCC can be resolved by using a sensitive and automated quantitative immunohistochemistry (IHC) technique that eliminates artifacts associated with manual scoring of biomarker expression. Our study is the first to use fluorescent IHC and automated quantitative analysis (AQUA) for measuring the expression of apoptotic proteins in OSCC. This high-throughput technique eliminates observer bias and provides reliable and reproducible estimates for biomarker expression. AQUA also provides essential measures of quality control that are absent in manual biomarker scoring techniques. We believe that the robustness and reliability of AQUA in measuring biomarker expression will help address the contradictory results regarding the prognostic significance of apoptotic biomarkers in the current literature. Finally, AQUA can measure protein expression in distinct cellular compartments thereby providing valuable insight into cancer mechanisms and tumor biology at a cellular level.

The inclusion of normal oral cavity squamous epithelium (OCSE) in our study demonstrates important differences in subcellular localization of apoptotic proteins in normal versus cancer tissue. We report that high Bax expression is independently associated with improved prognosis in OSCC. Therefore, measurement of Bax expression by AQUA can be adapted into a simple IHC test that might be useful for treatment selection and prognostication.

Our study, performed in a uniformly treated cohort of OSCC patients reduces biases associated with treatment heterogeneity and tumor-site-specific variability, thus increasing the utility of our results. Although we performed our study in a cohort of
OSCC patients, the AQUA technique described in the study could be used to improve the assessment of apoptotic protein expression in all other tumor-types. We believe that the robust automated quantitative IHC technique, the subcellular localization analysis and the prognostic significance of apoptotic biomarkers in OSCC, described in this study, will be of great interest to the discerning readership of BMC Cancer.

Yours sincerely,

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Competing interests:

This study was funded by the Ohlson Research Initiative. The corresponding author (JCD) is the Director of the Ohlson Research Initiative. This does not alter our adherence to all BMC Cancer policies as detailed in the guide for authors. All other authors declare no conflict of interest.

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