Author's response to reviews

Title: CDO1 Promoter Methylation is a Biomarker for Outcome Prediction of Anthracycline Treated, Estrogen Receptor-Positive, Lymph Node-Positive Breast Cancer Patients

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Version: 3 Date: 4 May 2010

Author's response to reviews: see over
Dear Mrs. Leigh, dear Dr. Cox,

Thank you very much for reviewing our revised manuscript. We appreciate your remarks and have addressed your comments as described below point by point.

We hope that our current revision is suitable for publication in BMC Cancer. Feel free to contact us if you have any concerns.

We look forward to hearing from you.

Kind regards,

Dimo Dietrich

Regarding reviewer 1’s comments below,

- Comprison of the three previously identified methylation markers (BMP4, FGF4, and C20orf55) with CDO1.

I think that the author's should include a brief comparison of the results from the two studies in the discussion section, for example the relative strengths of the effects.

**Author Reply 1:** *We have added the following paragraph to the result section: “A subset of the patient samples (n = 136) were also included in a previous microarray study, where DNA methylation of BMP4, FGF4, and C20orf55 was identified as biomarkers for outcome prediction [14]. The p-values obtained by the log rank test in Kaplan-Meier survival analysis revealed comparable clinical performance of the CDO1 methylation biomarker in this subgroup as compared to FGF4 (CDO1 p = 0.0017, FGF4 p = 0.0030). BMP4 and C20orf55 were not significant in this small subgroup of patients (C20orf55 p = 0.4948, BMP4 p = 0.1100). The median DNA methylation score from the 136 patient samples was used as the cut point for patient stratification.”*

- CDO1 methylation in untreated lymph node-negative hormone receptor-positive breast cancer cohort in order to test whether CDO1 methylation is a marker referring general poor prognosis.

I agree with the reviewer that it would greatly improve the paper to include data on the untreated group of patients. The authors should include such data if they have it.

**Author Reply 2:** *We agree with the reviewers that it would be nice to include data on patients who have not received adjuvant treatment allowing us to determine the prognostic*
value of CDO1 methylation. However and unfortunately, data from such a cohort is currently not available so we cannot provide these data at this moment.

Regarding Reviewer 2’s comments,

1) Clarify whether the p-value obtained for CD01 on the validation set was corrected for multiple comparisons

I agree with the reviewer that the final p-value for CD01 should be corrected for 6 comparisons for the 6 biomarkers tested in the validation set. This is easy to do and the CD01 result still remains significant after a Bonferroni correction for 6 tests.

**Author Reply 3:** We have added the following sentence to the result section: “The result for CDO1 still remained significant after a Bonferroni correction for 6 tests (p = 0.0060).”

2) Provide additional supplementary information

I agree with the reviewer that it would be straightforward for the authors to include supplementary data on the 37 genes which showed some differential methylation, and this would be useful data for others in the field.

**Author Reply 4:** We have provided the respective supplementary data (additional file 2).

Additional minor points:

page 5 second line: Certainly in the UK breast cancer is the most common cancer regardless of gender so the authors should clarify what country they refer to when they state it is "second overall".

**Author Reply 5:** We have replaced this statement using a more recent publication and clarified which year and which country the data refer to (USA 2009).

page 5 second para:
.."if a biomarker was available” rather than "would be available"

**Author Reply 6:** We have corrected the sentence accordingly.

page 10 the second paragraph repeats the results section rather, and I do not think it is needed.

**Author Reply 7:** We have shortened the paragraph accordingly.