Reviewer’s report

Title: NDN and CD1A are novel prognostic methylation markers and differ by HPV status in patients with head and neck squamous carcinomas

Version: 2 Date: 21 July 2015

Reviewer: Nina McCarthy

Reviewer’s report:

Please find my below my comments regarding the lack of correction for multiple testing in this manuscript. The manuscript describes the association of the methylation status of six candidate genes with tumour recurrence and survival of head and neck squamous carcinomas in a cohort of 346 HNSCC patients.

Currently, there is no adjustment for multiple testing described in this study, despite the fact that six different genes and two different (but related) outcome measures (recurrence and survival) were examined. It is my opinion that the authors should address the issue of multiple testing in the manuscript and provide appropriate adjustment of the results presented.

It is not clear to me from reading the manuscript in its current form how many methylation sites in the promoter region of each gene were assayed in this study, and whether methylation at each individual CpG site or a summary value for methylation across each promoter was tested. If one summary value of methylation across each promoter was used (similar to the gene methylation index (MtI) described in reference 40, Shaw et al), then each gene can be viewed as a single unit, and six independent tests should be corrected for. If this was not the case and multiple methylation sites within each gene were examined individually, further correction may be necessary.

The authors should also comment on whether the outcomes examined in this study (recurrence and survival) constitute independent measures, and justify whether further adjustment is necessary (i.e. 6 genes x 2 outcomes = 12 independent tests).

Level of interest: An article of importance in its field

Quality of written English: Acceptable

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

Declaration of competing interests:

I declare that I have no competing interests