Author's response to reviews

Title: B7-H3 expression in colorectal cancer: associations with clinicopathological parameters and patient outcome

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Author's response to reviews: see over
To
Ms. Cherry Batad
The Journals Editorial Office,
BMC Cancer

Date: July 18th, 2014

Dear Editor,

We are pleased that you find our manuscript “B7-H3 expression in colorectal cancer: associations with clinicopathological parameters and patient outcome” suitable for publication in BMC Cancer.

Please find enclosed a new version of the manuscript (with changes in red), and see below for our comments regarding the REMARK checklist.

Sincerely,

Vibeke A. Ingebrigtsen

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Remark checklist

INTRODUCTION
1 State the marker examined, the study objectives, and any pre-specified hypotheses
This is covered in the introduction of the paper.

MATERIALS AND METHODS
PATIENTS
2 Describe the characteristics (for example, disease stage or co-morbidities) of the study patients, including their source and inclusion and exclusion criteria
The clinical and pathological features for the study patients are summarised in Table 1. Their source and inclusion and exclusion criteria are described in the “Patient cohort and tissue microarray” section.

3 Describe treatments received and how chosen (for example, randomized or rule-based).
Colon cancer patients received adjuvant chemotherapy when indicated according to the current recommendations from the Norwegian Directorate of Health. In general 5-FU based chemotherapy regimens were given. Likewise, rectal cancer patients received preoperative radiotherapy when indicated according to the current recommendations from the Norwegian Directorate of Health.

SPECIMEN CHARACTERISTICS
4 Describe the type of biological material used (including control samples) and methods of preservation and storage
This is covered in the “Patient cohort and tissue microarray” and “Immunohistochemistry” parts of the paper, however we have now specified that the positive controls were also from formalin-fixed and paraffin-embedded tissue.

ASSAY METHODS
5 Specify the assay method used and provide (or reference) a detailed protocol, including specific reagents or kits used, quality control procedures, reproducibility assessments, quantitation methods, and scoring and reporting protocols. Specify whether and how assays were performed blinded to the study endpoint.
This is covered in the “Immunohistochemistry” section of the paper.

STUDY DESIGN
6 State the method of case selection, including whether prospective or retrospective and whether stratification or matching (for example, by stage of disease or age) was used. Specify the time period from which cases were taken, the end of the follow-up period, and the median follow-up time.
The method of case selection is described in the “Patient cohort and tissue microarray” section, and we have now specified that the material was prospectively collected and that registration was controlled against the Norwegian Cancer Registry, to ensure that no cases were missing.
We believe that it is apparent from the study type and the current description that stratification or matching was not used.
The time period, the end of the follow-up period and the median follow-up time are also described in the “Patient cohort and tissue microarray” section.

7 Precisely define all clinical endpoints examined
This is covered in the last paragraph in the “Patient cohort and tissue microarray” section.

8 List all candidate variables initially examined or considered for inclusion in models
All candidate variables are listed in Table 1. Age was also registered and is described in the “Patient cohort” section.
9 Give rationale for sample size; if the study was design to detect a specific effect size, give the target power and effect size.
The sample size is based on the number of patients which underwent primary surgery for colorectal cancer at Oslo University Hospital Aker between 1993 and 2003.

STATISTICAL ANALYSIS METHODS
10 Specify all statistical methods, including details on any variable selection procedures and other model-building issues, how model assumptions were verified, and how missing data were handled.
The statistical methods are described in the “Statistical analysis” paragraph in the “Material & methods” section. No complex models were used, and model building issues are thus not relevant.

11 Clarify how marker values were handled in the analyses; if relevant, describe methods used for cutpoint determination.
This is covered in the final paragraph in the “Immunohistochemistry” section.

RESULTS
DATA
12 Describe the flow of patients through the study, the number of patients included in each stage of the analysis (a diagram may be helpful) and reasons for dropout. Specifically, both overall and for each subgroup extensively examined report the number of patients and the number of events.
This is described in the “Patient cohort and tissue microarray” section, however, we have now specified how many patients that died during the follow-up period. Furthermore, the number of patients at risk (ie; not censored or diagnosed with disease recurrence/dead) at each time point is given below the Kaplan-Meier curves in Figure 2 and 3.

13 Report distributions of basic demographic, characteristics (at least age and sex), standard (disease-specific) prognostic variables, and tumor marker, including numbers of missing values.
This is covered in the Supplementary table.

ANALYSIS AND PRESENTATION
14 Show the relation of the marker to standard prognostic variables.
The relation of the marker to standard prognostic variables are described in section, and summarised in Table 2.

15 Present univariable analyses showing the relation between the marker and outcome, with the estimated effect (for example, hazard ratio and survival probability). Preferably provide similar analyses for all other variables being analyzed. For the effect of a tumor marker on a time-to-event outcome, a Kaplan-Meier plot is recommended.
Univariate analyses for the tumor marker and other variables are presented in Table 3. Kaplan Meier plots are shown in Figure 2 and 3.

16 For key multivariable analyses, report estimated effects (for example, hazard ratio) with confidence intervals for the marker and, at least for the final model, all other variables in the model.
Univariate analyses did not display significant associations between B7-H3 expression and patient outcome in CRC patients (Table 3), except for the strong association between the presence of nuclear B7-H3 expression and reduced recurrence-free survival in TNM stage I patients (p = 0.006, Fig. 3). However, this association was not seen when using overall survival as an endpoint. Proceeding with multivariate analysis where considered unnecessary as the clinical significance of our finding was rather uncertain.
Among reported results, provide estimated effects with confidence intervals from an analysis in which the marker and standard prognostic variables are included, regardless of their statistical significance. Table 3 shows the associations between survival and standard clinicopathological parameters and B7-H3 expression. As the main finding in our paper is that nuclear B7-H3 was not a strong prognostic biomarker in colorectal cancer patients, we did not continue with multivariate analyses.

If done, report results from further investigations, such as checking assumptions, sensitivity analyses, and internal validation. This is not applicable, please see our comment above.

DISCUSSION

Interpret the results in the context of the pre-specified hypothesis and other relevant studies; include a discussion of limitations of the study. This is covered in the discussion section of the paper.

Discuss implications for future research and clinical value. This is covered in the discussion.