Author’s response to reviews

Title: Prognostic and predictive value of cathepsin X in serum from colorectal cancer patients

Authors:

Tjasa Vizin (tjasa.vizin@ffa.uni-lj.si)
Ib J Christensen (ib.jarle@finsenlab.dk)
Michael Wilhelmsen (michael.wilhelmsen@regionh.dk)
Hans J Nielsen (h.j.nielsen@ofir.dk)
Janko Kos (janko.kos@ffa.uni-lj.si)

Version: 2
Date: 24 March 2014

Author’s response to reviews: see over
Dear Editor,

We re-submit our paper (1371187394119453) entitled “Prognostic and predictive value of cathepsin X in serum from colorectal cancer patients” to be re-considered for publication in BMC Cancer. We have considered the comments, concerns and suggestions of both reviewers and provided additional explanation and changes to improve the manuscript.

Modifications in response to the reviewer’s comments were made in the revised manuscript and the list of the changes and explanation to the comments is the following;

**Reviewer #1**

*In this manuscript, the authors set out to validate earlier results on the prognostic value of serum Cat-x in a limited number of colorectal patients. No difference in serum Cat-x is found between controls and CRC patients, and no difference in survival between patients with high or low Cat-x serum levels. However, further analyses revealed that Cat-x was prognostic within the group with resectable, stage I-III, disease, and that this prognostic value was only present in patients that did not receive chemotherapy. The authors conclude that although the validation was negative, the present results should be confirmed in prospective studies.*

**Major remarks:**

*As often with this kind of studies, the statistical methods are important. In this manuscript, the authors report values as mean +/- SD, suggesting normal distribution of values, but also employ Spearman rank correlation tests, a non-parametric test. Similarly, Cat-x is entered as a continuous variable in Cox regression, but only after log2 transformation. Why was this done? Was this to normalize Cat-x values? If so, values should not have been reported as mean +/-SD. On the other hand, from Table 2 it seems that Cat-x levels are normally distributed. The authors should more clearly describe which test was done and why. E.g., on page 9 they report multivariate analyses including interaction terms, but in the statistics section of the M&M section this is not explained, or at least not in sufficient detail.*
Cathepsin X was log transformed in order to achieve linearity between the log hazard and the marker. This assumption was assessed using martingale residuals as stated in the Statistical analysis. Log transformation base 2 has the additional advantage that the hazard ratio is for a two fold difference in marker level.

Values of cathepsin X in Table 2 have been reported as mean +/-SD as an initial information. For further analysis the log transformed values have been used.

The inclusion of interaction terms has been added to the Statistical analysis on page 8 (1st paragraph). The part on the log rank test, which was used to test the difference between the strata, was moved from the Statistical analysis on page 8 to Figure 1 legend on page 19.

The Kaplan-Meier estimates of survival probabilities are mainly for illustration and not statistical inference, therefore the statistics section has been revised with less emphasis on the statistical differences between the strata.

In line with the previous comment, on page 10 the authors report how they used median-dichotomized patients, with or without chemo, and then between tertiles, and stratified for stage, i.e. I-III or III alone etc. They do these analyses in both univariate and multivariate analyses. Overall, this large number of analyses would suggest that this study might suffer from a large chance of false-positive results, especially as the authors do not correct for multiple testing. I would strongly suggest to limit the number of statistical tests, and the number of variables in multivariate analyses.

The primary analysis in this study is the multivariable analysis and in our view we have a sufficiently large number of events for the conclusions drawn. The pre-specified hypothesis was that cathepsin X was associated to outcome although the interaction was not anticipated. A correction for multiple testing would still result in cathepsin X being significant.

Can the authors hypothesize on the origin of Cat-x in controls? It would seem strange that serum levels are similar in patients and controls, while on the other hand Cat-x is informative on survival of patients.

Cathepsin X is secreted from tumor and immune cells and its overexpression and secretion is associated with the progression of disease. Molecular targets and mechanisms linking cathepsin X with progression of cancer are well presented in Discussion, 3rd paragraph. With regard to nonmalignant cells, cathepsin X is abundantly present in monocytes and macrophages and can be released into the circulation of healthy persons due to basic and regulated secretion by leukocytes during normal physiological immune cell activation and turnover.
In cancer patients, increased secretion of procathepsin X could reflect its overexpression in tumour and tumour associated immune cells, as well as other immune cells. Subsequently, cathepsin X is obviously higher in patients with higher risk compared to those with better prognosis. On the other hand, cathepsin X seems to be involved in response to chemotherapy: its levels after therapy are consequently changed and are not correlating with survival anymore. Chemotherapeutics may interfere with the molecular targets of cathepsin X, and with the processes involving its enzymatic and non-enzymatic action and may therefore regulate also its expression and secretion.
We added these additional explanations to pages 12 (2nd paragraph) and 13 (1st paragraph) of the manuscript.

Minor remarks:

On page 8, the authors state that they used Cox regression analyses to assess the “association of total Cat-x with overall survival and other clinicopathological parameters.” The authors probably mean “association of total Cat-x and other clinicopathological parameters with overall survival.”

This part has been corrected in the manuscript (page 8, Statistical analysis, 1st paragraph).

Page 13: what is a »breast cancer expressing mice model«

A breast cancer expressing mice model in the manuscript is referred to a transgenic mouse model for metastatic breast cancer, which is induced by a polyomavirus middle T oncogene (PymT) [1, 2]. In the study by Sevenich et al. [2] to which we are referring in the manuscript, they used a transgenic mouse model harboring a transgene for the mammary duct-specific expression of PymT and analyzed the effects of single and combined cathepsin B and cathepsin X deficiencies on breast cancer progression.


Reviewer #2

9. I am not competent in evaluating quality of English in articles, however in conclusions I propose considering the following text: Our results do not confirm the association of cathepsin X with overall survival for the entire set of 264 colorectal patients but only for patients in stages I-III with local resectable disease. Moreover, a significant association of cathepsin X with overall survival within a group of patients in stages I-III, who did receive chemotherapy suggest a possible predictive value for response to chemotherapy, in this group of patients, which has to be confirmed in a further study.

This part has been revised in the manuscript Abstract Conclusions on page 3.

At page 8, 3rd line from the bottom „ envoriment” should be changed to „environment”?

We have corrected it in the manuscript (page 8, 3rd line from the bottom).

In References at the 27, 31 and 43th positions capital leters should be changed
to small letters. eg. in Wang J, Chen LL, Li Y, Guan XY: Overexpression of
cathepsin Z contributes to tumor metastasis by inducing epithelial-mesenchymal

We have corrected it in the manuscript (pages 17 and 18; References 27, 31, 34 and 43).

We hope that the responses to the comments of the reviewers and the additional explanations and
corrections included in the manuscript make it acceptable for publication in BMC Cancer.

Sincerely,

Janko Kos, Ph.D.

On behalf of authors