**Author's response to reviews**

**Title:** Serum levels of Dickkopf-1 are an independent prognostic marker in prostate cancer

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**Version:** 3  
**Date:** 12 August 2014

**Author's response to reviews:** see over
August 12, 2014

Dear Dr. Ratin:

We thank the reviewers for their constructive comments. Enclosed, please find our revised manuscript entitled "High serum levels of Dickkopf-1 are associated with a poor prognosis in prostate cancer patients". We have revised the manuscript and responded to all comments as specified below. We hope that this manuscript is now acceptable for publication.

Sincerely yours,

Tilman D. Rachner, MD
Reviewer: 1

We thank Professor Gavriatopoulou for her helpful comments.

Discretionary revisions:
Although the results of the study are of potential interest, the main question to be answered is if and in what way anti-DKK-1 might be effective in patients with high levels of serum DKK-1 and especially in which selected subgroup (high risk patients-extended disease).

We agree with the reviewer that this is an important question when thinking about the use of potential anti-DKK-1 treatments in the clinical setting. However, large interventional studies will be needed to answer this question. We have included studies that address potential mechanisms by which DKK-1 may affect tumor biology and expanded the discussion on these thoughts (see page 6-7).

There are no data whether all the TMAs and serum samples were collected and stored under the same conditions.

All serum and tissue samples were collected in a standardized fashion and were stored under the same conditions. This has been included in the material and methods section.

Minor essential revisions:
How was TMA staining score defined?

This information is included on page 4 and has been extended (p4, line 104-106)

A table with patients characteristics only for those with serum sampling should be provided. Were these patients randomly selected? What were the inclusion criteria of the patients included in the study? Pathology characteristics regarding the type and aggressiveness of the tumor as well as the staging of the tumor were not provided.

Patients included in the serum analyses were randomly selected according to availability of serum material. Detailed information for this cohort of patients is included in table 2. Information provided includes age, staging, PSA and Gleason score. Importantly, there is no significant difference on these characteristics between patients with high or low DKK-1.

Major compulsory revisions: Considering the high incidence of prostate cancer in order to provide safe and powerful results more serum samples should be evaluated and that I believe is the main weakness of this study. Furthermore the population should be more clearly selected and described in order to avoid the possible heterogeneity within patients with prostate cancer.

We agree with the reviewer that the number of serum samples assessed from patients in this study is a limiting factor. Unfortunately, we did not have access to a larger number of serum samples from affected patients. We have emphasized this limitation and acknowledged the need for larger trials to further validate our finding in the discussion.

The data is sound enough although the title of the study should be more accurate.

We have adapted the title to "High serum levels of Dickkopf-1 are associated with a poor prognosis in prostate cancer patients" and hope that this is in agreement with the reviewer.
Reviewer: 2
We thank Professor Terpos for his helpful comments.

1. I would like the authors to provide values of Dkk-1 for healthy subjects of similar age and gender with the patients as there may be significant overlapping in Dkk-1 values; something that can reduce the value of the finding for the every-day clinical practice.

We agree with the reviewer that this is an important point. DKK-1 values are known to have a large variability and overlapping serum levels have been previously described in other malignancies like breast cancer and myeloma.

To address this question in prostate cancer patients, we have performed DKK-1 serum measurements in a control cohort of 24 male patients with benign prostate hyperplasia (BPH). This group did not significantly differ in gender or age from our prostate cancer group. As expected they had lower levels of PSA. In our assessment we found a significant overlap of DKK-1 values 25.3±6.0 (n=23) vs 27.9±12.9 (n=80, all patients with prostate cancer). While this finding may limit the diagnostic value of DKK-1 as a prognostic marker for the detection of prostate cancer (a notion we already put forward in the manuscript) it does support our hypothesis that non-tumor derived DKK-1 may influence tumor biology to a larger extent than previously suggested. This finding has been included and is discussed in the manuscript.

2. Do the authors have any information for the bone status of the patients in the two groups? i.e. male osteoporosis, osteoarthritis, etc. The number of patients with bone metastases in both groups (Dkk-1 low and high) has to be clearly reported.

At the time of blood sampling all patients included in this study were considered to be M0 defined by standardized tumour staging prior to prostatectomy. The fact that none of these patients had detectable bone lesions has been mentioned in the text. Unfortunately, we do not have data on bone mineral density for any of these patients.

3. Finally, can the authors suggest a Dkk-1 level for validation in larger series of patients?

The authors acknowledge the need for validation in larger cohorts of patients. However, defining a DKK-1 level would be somewhat difficult regarding the variability of DKK-1 levels reported in the literature depending on assay type and conditions.

Reviewer: 3
We thank Professor Thalmann for his helpful comments.

Major compulsory revisions:

1) The authors report on DKK-1 expression as a prognostic marker. In the TMA DKK-1 was not prognostic. The fact of the high expression in the adjacent tissue needs some discussion. Why? What does this mean? How about bone metastases?

2) Why would DKK-1 in the serum be predictive and not in the tissue? Are there splice variants? A western blot would eventually provide more information.

Point 1 and 3 both address similar aspects, we would therefore like to address these points together.

The question why DKK-1 serum levels are predictive and DKK-1 tissue levels are not is one of the interesting questions that arise from this paper. One explanation would be that the increased levels of DKK-1 in the tumor are not the major contributor to DKK-1 serum levels, an idea which would be supported by the finding that DKK-1 serum levels do not directly
correlate with DKK-1 prostate cancer expression. If this is the case, it may be non-tumour derived DKK-1 that affects and influences the cancer biology. These ideas have been extended in the manuscript. To our knowledge there are no known splice variants of DKK-1.

We do not have information on the occurrence of bone metastases in the follow up of these patients at this time.

2) For the serum test the sample size seems somewhat small. What was the power calculation?

We agree with the reviewer that the relatively small number of serum samples is a limiting factor of the study. This limitation is mentioned in the discussion. Unfortunately we did not have access to a larger number of samples. Furthermore, as we did not have any experience of DKK-1 serum assessment in prostate cancer patients, a power calculation prior to performing this experiment would not have been possible due to the lack of mean values and standard deviation.

4) The manuscript is descriptive and shows no functional data that would strengthen the authors point.

We agree with the reviewer that our study is descriptive. This point has been emphasized in the manuscript. However, we have included a number of excellent preclinical studies on DKK-1 and prostate cancer biology (namely references 8-10) that clearly define DKK-1 as a modulator of prostate tumor biology. Thus, we believe that the results of our study using a large, well-defined cohort of prostate cancer patients are of valuable clinical information to put the experimental and clinical data into context.