Author's response to reviews

Title: Circulating endothelial cells are an early predictor of tumor response in renal cell carcinoma patients treated with sunitinib

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Author's response to reviews: see over
Dear Mr. Cochrane.

We appreciate the reviewer’s comments on our paper and addressed the issues raised in the peer reviewing process. Please find a point-by-point annotation given for each reviewer’s comment.

An issue raised by all reviewer was the labeling of figures and legends, which was introduced during erroneous upload of the figures. Figures have now been re-assembled and uploaded in the correct format, displaying now figures with corresponding legends.

**Reviewer 1 (Glen Kristiansen):**
Issues according to figures have been addressed above.
The *desperate need of biomarkers* has been changed to *desperately needed*, as suggested.

**Reviewer 2 (Glenn Kroog):**
A) Issues according to figures have been addressed above.
B) We included a paragraph on statistics which indicates its use in our study in the material and methods section. As mentioned by the reviewer patients have come off study for progression, which introduces selection bias into tests which assess changes over time. We therefore changed analyses shown in Fig. 2A to a t-test of peak values after 14 and 28 days of treatment compared with baseline.
C) As already assumed by the reviewer, responders were defined by achieving either objective response or stable disease as best response during therapy. We added these definitions to the methods section.

**Reviewer 3 (Paul de Souza):**
Response assessment and definition for responders and non-responders has been added to the methods section.

For each patient CEC, monocytes and sVEGFR2 were evaluated in parallel. Four patients who were included for the initial assessment of monocytes were excluded from current analyses due to missing values of CEC.
Statistics were recalculated accordingly and updated within the manuscript. However, our conclusion remained unaffected from these changes.

We agree on the reviewer’s assumptions in regard to the possibility of selection bias, which cannot be excluded for samples reflecting changes over time and different outcome to treatment. This has been added to the discussion. However, this error does not apply for the assessment of monocytes and sVEGFR2 at baseline, which were significant in responders and non-responders and indeed may predict response to sunitinib.

The possibility of myelosuppression has been added to the discussion.

We do recognize the reviewers doubts in regard to the usefulness of CEC, sVEGFR2 and monocyte counts for treatment with sunitinib. However, we do believe that we always discussed these findings very thoroughly and assessed the need for validation studies - as mentioned within the last paragraph of the discussion section. We now added a discussion about the limitation of our study, according to the reviewer’s comments. Given all limitations of our study it nevertheless is supported by similar findings of 2 other independent studies assessing CEC as a potential biomarker in patients treated with sunitinib (cited in paper: Vroling et al. [23]; Norden-Zfoni et al. [4]). Furthermore, some limitations mentioned above may not apply for the assessment of biomarkers prior to drug exposure. We therefore concluded that CEC and baseline assessment of sVEGFR2 and monocyte counts may be useful and need additional prospective confirmation, which to our believe is rational to conclude without over-interpretation.

Issues with figures have been discussed above.

Discrepancy of the patient cohort for monocyte assessment has been addressed above. In summary, 4 patients were excluded from monocyte analysis due to missing CEC measures.

Reviewer 4 (James W Mier):
none

We do acknowledge the reviewer’s comments on our study and addressed the criticism raised during the peer-review process accordingly. We believe that our manuscript improved during this process substantially, which is why we now re-submit our article for further assessment for publication.

Sincerely,
Yours,

Dr. med. Viktor Grünwald