Author's response to reviews

Title: Prognostic information of serial plasma osteopontin measurement in radiotherapy of non-small-cell lung cancer

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Author's response to reviews: see over
Dear Sirs,

we hereby submit our revised manuscript “Prognostic information of serial plasma osteopontin measurement in radiotherapy of non-small-cell lung cancer” for consideration of publication in *BMC Cancer*.

The manuscript has been entirely revised and all changes in the original manuscript have been highlighted in yellow in the text.

The highly helpful reviewer comments have been thoroughly addressed and a point-by-point description of the changes and revisions is given below including individual responses to each comment.

Sincerely,

(Christian Ostheimer)  (Prof. Dr. Dirk Vordermark)
**Descriptions of changes and response to the reviewer’s comments:**

**General:**

- Line numbers have been included in the entire manuscript to allow easier review.
- Minor essential revisions of both reviewers: the entire manuscript has been revised for grammar and English language. Spelling errors, typos and sentence structure have been edited for clarity and the manuscript was shortened in the introduction, results section and discussion where appropriate.
- References have been updated: initial reference 33 and 34 has been replaced by two new references and initial reference 28 and 29 have been re-numerated.

**Response to reviewer Blasberg’s comments:**

The reviewer raised some minor essential and discretionary revisions which have all been addressed in the revision.

The reviewer mentioned several text passages and lines which needed corrections:

- Line 120-125 *(initially line 121-123)* has been re-written for clarification
- Line 211-215 *(initially line 215)* has been corrected
- Line 247-249 *(initially line 242-243)* has been edited for clarity
- Line 261 and 265 *(initially line 255)* has been revised
- Line 341 *(initially line 327)* has been shortened
- The sentence, initially line 384, has been shortened

- **Response to discretionary revision comment:**

Since time points for OPN assessment were fixed in this analysis (i.e. before, at the end and four weeks after radiotherapy), the three groups, as defined by rising, stable and falling OPN plasma levels over time, reflect three groups with distinct OPN velocity. Calculation of parameters such as OPN change per day or months would lead to essentially the same grouping. Specific calculation of OPN velocity appears very attractive in larger future studies.

**Line 423/424 has been included in the manuscript to address the reviewer’s comment.**
Response to reviewer Zips’s comments:

The reviewer raised three major compulsory and minor essential revisions which all have been addressed in the revision.

- **Response to reviewers 1st comment:**

  The reviewer asked for a clearer statement of the primary hypothesis that OPN assessment during and after radiotherapy might provide additional/superior prognostic information compared to baseline OPN detection.

  In this regard, the introduction (abstract, main text) was partly re-written to state the hypothesis of this study more clearly.

  Additional multivariate analyses have been carried out to test the prognostic quality of absolute pre-treatment (baseline) OPN plasma levels against that of relative post-treatment (t1 to t2) plasma level changes. Multivariate analysis demonstrated that baseline OPN plasma levels were not an independent prognostic predictor in this study which is in accordance with our previous study, where OPN only was an independent predictor of survival in the multivariate analysis if co-detected with other hypoxia-related proteins [15]. These results suggest that relative OPN plasma level changes after radiotherapy rather than absolute pre-treatment OPN (if detected as single marker) are prognostically relevant.

  The results section “OPN in an exploratory predictive model for overall survival” has been updated accordingly (line 295-297 and 305-307). The discussion has been edited for the new results (line 398-408).

- **Response to reviewers 2nd comment:**

  In his 2nd comment, the reviewer suggested that OPN plasma level changes might be a surrogate of tumor volume changes (given the association of OPN plasma levels with GTV for instance) and prognostic effects of OPN during or after radiotherapy.

  GTV is in part represented in T stage which was included in the multivariate model. However, line 385-396 was partly re-written to address this issue and to underline the potential benefit of integrating CT imaging at time points of OPN readings in future studies.
Response to reviewers 3rd comment:

The reviewer questions the relevance of post-treatment OPN plasma level changes for radiotherapy individualization which is a justified concern. However, in this and the previous study [15] not only OPN plasma level changes during and after radiotherapy but also absolute pre-treatment plasma levels were evaluated. While baseline OPN levels (indicating a hypoxic, aggressive and radioresistant cancer phenotype) could influence radiotherapy individualization by means such as hypoxic modification, which might be available in future, post-treatment OPN level changes (identifying patients with high risk for death and relapse after radiotherapy) could help in the decision-making process for ongoing cancer treatment after radiotherapy. We acknowledge that the time point at which the prognostically relevant OPN changes become apparent, i. e. at t2, is too late to individualize radiotherapy.

Line 425-441 has been included in the discussion section to address this issue.

Minor essential revisions:

The whole manuscript has been shortened and redundant text passages have been removed where appropriate. The entire manuscript has been revised for language.