Author's response to reviews

Title: Connexin 26 and 46 expression refines intermediate prognostic subgroups of residual tumor classifications in neoadjuvant treated breast cancers

Authors:

Ivett Teleki (telekiivett@gmail.com)
Tibor Krenacs (krenacst@gmail.com)
Marcell A Szasz (szaszam@gmail.com)
Janina Kulka (janinakulka@gmail.com)
Cornelia Leo (Cornelia.Leo@usz.ch)
Bärbel Papassotiropoulos (baerbelmaria@hotmail.com)
Cosima Riemenschnitter (cosimari@hotmail.com)
Holger Moch (holger.moch@usz.ch)
Zsuzsanna Varga (zsuzsanna.varga@usz.ch)

Version: 2 Date: 21 September 2012

Author's response to reviews: see over
Author's covering letter for initial submission

Title: Connexin 26 and 46 expression refines intermediate prognostic subgroups of residual tumor classifications in neoadjuvant treated breast cancers

Authors:

Version: 1 Date: 19 September 2012

Comments: see over
Dear Dr. Sabine Linn

Editor of BMC Cancer
MS: 1430923391755449

Dear Dr. Sabine Linn,

We are very grateful for the useful criticisms given to our manuscript of Teleki et al, entitled: “Connexin 26 and 46 expression refines…” by your reviewers and we made our revisions according to their comments.

Here we declare that the data in the manuscript is original and the manuscript is not under consideration elsewhere.

*Ivett Teleki and *Tibor Krenacs equally contributed to this work.

Barna Wichmann contributed to the statistical analysis of the revised manuscript thus he was involved into the authors list.

As requested by the editor:

The authors declare no conflict of interest concerning the content of this manuscript.

All authors read and approved the final manuscript.

Author’s contribution, included also at the end of body text:

ZsV initiated this study with TK and collected the tissue samples and their related clinicopathological data in co-operation with CL, BP, CR, HM who also treated the patients. TK supervised immunomorphological studies performed by IT and compiled the paper together with ZsV and IT. IT, MSZ and BW performed the statistics after scoring together with JK. JK also gave advice on the interpretation of the results.

We would greatly appreciate if you considered our revised version for publication in BMC Cancer.

Best regards:

Tibor Krenacs

Below you can find our point-by-point response to your comments and changes made to the original manuscript, which are shown underlined in the revised text:

To R1, A Jager

To major comment

As it was suggested we performed multivariate analysis (also included in the Materials, page 8) between features that preliminarily showed correlation with overall survival but not with each other. We summarized these data in Table 4, and in the last chapter of results (page 11). These were restricted to Cx26 in the whole patient population after chemotherapy and to Cx46 both prior to and after chemotherapy in the EWGBSP TR2b, Sataloff TB and the Miller-Payne G3 subgroups. The rest of the markers such as TNM, grade, Ki67 or HER2 expression pre-chemo tumor size and pathological response did not correlate with prognosis (though some showed strong tendency), therefore, they had to be ignored from multivariate analysis.

Minor comments

We have included an additional table on the relationships between connexin expression, cell proliferation and other clinicopathological parameters (Table 3). The text of the results was also supplemented with these data briefly (page 9).
The scoring scale between 0-10 for Ki67 represents percent thresholds of positive cells rendered evenly to the different categories between 0 to 100%. This was also specified in the methods (page 7) and Ki67 results added to Table 1 in the revised manuscript.

We understand the point of the reviewer to make all graphs along consistent thresholds, however, the correlation between Miller-Payne G3 and Cx46 expression post-chemo (lower right-most graph in Fig 6) was only significant at this modified threshold. If the editor finds this as a critical issue we can leave it out from this combined figure.

We refer to the limitations of this study at the end of the discussion related to the restricted number of cases analyzed and the need for further validation.

To R2. Marwan El-Sabban
Since connexins may act both as cell membrane gap junctions (hemichannels) and cytoplasmic protein-protein interactions we considered both of these sites at scoring. Further functional studies are needed to clarify these functions separately in breast cancer.
All data concerning the relations between connexin and Ki67 expression, as requested both by Reviewer 1 and the Editor, were included into the revised manuscript (Table 3 and page 9).

To the Editor
Standard chemotherapy protocols applied for the patients selected in this study are added to the Materials and Methods (pages 4 and 5).

Since we can not discriminate prognosis form prediction and tested our markers only against overall survival in relation to connexin expression we consistently use the word prognosis or assessment in the revised manuscript.

Correlations between connexin and Ki67 expression were included into the revision as it was also suggested by reviewer 1 (Table 3 and page 9).

Multivariate analysis was also performed in all possible correlations, however, as it was also mentioned to reviewer 1, some of these parameters did not correlate with survival, therefore, they had to be ignored from the analysis (Table 4 and the last chapter of results).

We have put an additional combined figure (Figure 1) showing changes of the expression of the 4 Cx isotypes (Cx26, -32, -43,-46) upon chemotherapy related to individual patients. Since Cx46 levels were changed at the individual patient’s level (thought it was not statistically significant in the whole group) we left out the sentence (after Figure 5B on page 12) “Since Cx46 levels do not seem to alter during the course of therapy”… from these sites of the revised manuscript.
However, this combined figure also revealed that the connexin isotypes which had negative correlation with prognosis (Cx26) or positive correlation with known negative prognostic markers (Cx32 vs HER2 level, or Ki67 index) were mainly reduced upon chemotherapy. On the other hand, Cx46 levels showing positive link with survival, increased. These findings may also reflect the efficiency of chemotherapy, which was stated in the revised manuscript (2nd paragraph on page 13)

The number of patients was included into the Kaplan-Meier graphs (Figures 3, 4 and 6).

A sentence on the limitation of the study related to the relatively low number of patients and the need for further validation was added to the end of discussion (page 14).