Author's response to reviews

Title: Nuclear Ep-ICD accumulation predicts aggressive clinical course in early stage breast cancer patients

Authors:

Gunjan Srivastava (gsrivastava123@gmail.com)
Jasmeet Assi (jasmeet.assi@gmail.com)
Lawrence Kashat (lkashat@gmail.com)
Ajay Matta (matta.ajay@gmail.com)
Martin Chang (MChang2@mtsinai.on.ca)
Paul G Walfish (pwalfish@mtsinai.on.ca)
Ranju Ralhan (rralhan@mtsinai.on.ca)

Version: 3 Date: 10 July 2014

Author's response to reviews: see over
July 10, 2014

To,

Prof. Paul van Diest
Executive Editor
BMC Cancer

Sub: Submission of revised manuscript entitled “Nuclear Ep-ICD accumulation predicts aggressive clinical course in early stage breast cancer patients”

Authors: Gunjan Srivastava, Jasmeet Assi, Lawrence Kashat, Ajay Matta, Martin Chang, Paul G. Walfish, Ranju Ralhan

Dear Prof. van Diest,

We are submitting the revised manuscript entitled “Nuclear Ep-ICD accumulation predicts aggressive clinical course in early stage breast cancer patients” on behalf of all the authors for consideration for publication in BMC Cancer.

The manuscript has been revised taking into account all the comments of both the reviewers.

The point-wise response of the authors’ to all the reviewers’ comments is also submitted for ready reference.

We are confident that the revised version of the manuscript will be acceptable and our findings will be of keen interest for the readers of BMC Cancer.

We look forward to a favorable response.

Sincerely yours,

Ranju Ralhan

(Dr. Ranju Ralhan)
Authors’ response to the Reviewers’ reports

Title: Nuclear Ep-ICD accumulation predicts aggressive clinical course in early stage breast cancer patients

Reviewer's report-I

This is a well written paper with interesting results for the scientific community. However, some items need to be addressed.

We thank the reviewer for the favorable review and interest in our work.

Major revisions:

1. Kaplan Meier curves show difference in survival depending on nuclear staining. However, as the right panel is a fairly homogeneous group of invasive breast tumors, the group as a whole, left panel is a mixture of different types of carcinomas, even including in situ carcinomas. This is not right. The author has to show the kaplan meiers for each of the subgroups and after multivariate analysis.

Authors’ Response: Kaplan Meier analyses have been done for DCIS and IDC cases separately and the results are given in Figure 3A &B and Table 4. See page 14 lines 14-18.

2. The paper is based on nuclear protein expression, however the biological meaning of this localisation is not explained.

Authors’ Response: The biological significance of nuclear Ep-ICD localization has been explained in the discussion. See page 15 lines 22,23 and page 16 lines 1-5.

Minor points.

In the M&M section: it's unclear how the regions of most aggressive growth are selected. Explain in the text.

Author’s Response: Immunopositive staining was evaluated in five areas of the tissue sections representing the highest tumor grade (Nottingham system); See page 11 lines 5-7.

Reviewer's report-II

This manuscript describes the predictive value of nuclear, cytoplasmic and membranous EpCAM expression and concludes that nuclear EP-ICD positive patients had a poor
prognosis. The manuscript is well written and the conclusions are potentially relevant for risk stratification in breast cancer patients. However, I have two major and several minor comments.

**Major comments:**

1. The study includes 61 patients with DCIS and 1 patients with LCIS. These patients have a survival rate close to 100%, so these cases should not be included in the survival analysis. These cases could be mentioned but they should not be analyzed together with the invasive breast cancers.

   **Authors’ Response:** Kaplan Meier analyses have been done for DCIS and IDC cases separately and the results are given in Figure 3A &B and Table 4. The LCIS patient has been removed from analysis. See page 14 lines 14-18.

2. The authors claim to have characterized different subtypes of breast cancer. However, the study mainly included IDC patients (without known Her2 status) and very few ILC and IMC cancers, so the results are based mainly on IDC cases. First, using the wording ‘all patients including IDC cases’ in the method, result- and discussion section is confusing. Why not just use ‘all breast cancer patients’?

   **Authors’ Response:** We agree. As suggested, all patients including IDC cases’ in the method, result- and discussion section has been replaced by ‘all breast cancer patients’.

3. Second, the limitation of the very small numbers of breast cancer histotypes should be mentioned in the discussion.

   **Authors’ Response:** The limitation of the very small numbers of breast cancer histotypes has been mentioned in the discussion. See page 18 lines 19-20.

4. The sentences in the discussion: ‘our study is the first in-depth characterization of EP-ICD expression in different subtypes of breast cancer’ and ‘nuclear Ep-ICD is detected in all subtype of breast cancer’ should be changed, since these conclusions cannot be made based on the patient selection.

   **Authors’ Response:** These sentences have been revised.
Furthermore, our study is the first in-depth characterization of Ep-ICD expression in IDC of the breast. See page 15 lines 21-22.

In conclusion, nuclear Ep-ICD was detected in DCIS and IDC and found to be associated with recurrence in these patients. See page 19 lines 7-8.

Minor comments:
1. Background: explain abbreviations: HRneg/Tneg
   
   Authors’ Response: hormone receptor negative and/or triple negative breast tumors. See page 7 lines 6-9.

2. Methodes sections; evaluation of IHC and scoring: explain how the most pathologically aggressive areas are defined.
   
   Authors’ Response: The term ‘most pathologically aggressive areas’ has been replaced with areas of the tissue sections representing the highest tumor grade (Nottingham system). See page 11 lines 5-7.

3. Numbers of ILC and IMC cases are too small to give percentages.
   
   Authors’ Response: We agree. Percentages have been deleted in the revised manuscript. See page 13 lines 5-11.

4. Results: Nuclear Ep-ICD overexpression was significantly associated with early tumor grade (grade I and II). This should be low or intermediate tumor grade instead of early tumor grade, since in breast cancer there seems hardly any progression in grade (there is no progression from low grade to high grade invasive ductal carcinoma). The same holds true for the conclusion section (it is possible that nuclear Ep-ICD accumulation is an early indicator of tumor progression, as evidence by its correlation with low grade).
   
   Authors’ Response: Early tumor grade (grade I and II) has been replaced with be low or intermediate tumor grade in the revised manuscript. See page 13 lines 18-21.

5. Explain why Her2 status is not included in this study
Authors’ Response: In this retrospective study, breast cancer cases were selected for the period 2000-2007. The hospital records did not have data on HER2 status for many of the patients. Hence this information could not be included in our study. (See page 9 lines 21-22).

6. In the conclusion it is mentioned that 50/75 nuclear Ep-ICD positive IDC patients did not have a recurrence, limiting the role of using this biomarker for risk stratification. This result should also be mentioned in the result section of the manuscript.
Authors’ Response: This result has been mentioned in the result section of the manuscript. See page 15 lines 1-2.

7. Abstract: explain abbreviation of IDC (invasive ductal carcinoma)
Authors’ Response: Invasive ductal carcinoma (IDC) has been added in the abstract. See page 4 lines 15-16.