Reviewer's report

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

Version: 2
Date: 1 June 2014

Reviewer: carolien van deurzen

Reviewer's report:

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 patient 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.

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’?
Second, the limitation of the very small numbers of breast cancer histotypes should be mentioned in the discussion. 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 conclusion cannot be made based on the patient selection.

Minor comments:
1. Background: explain abbreviations: HRneg/Tneg
2. Methodes sections: evaluation of IHC and scoring: explain how the most pathologically aggressive areas are defined
3. Numbers of ILC and IMC cases are too small to give percentages
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).

5. Explain why Her2 status is not included in this study.

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.


**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests.