Reviewer's report

**Title:** HTF9C expression is prognostic in her2+ breast cancer

**Version:** 1  **Date:** 14 May 2009

**Reviewer:** Ergin Kilic

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The overall aim of the presented study is to find markers which allow a stratification of the Her2 positive invasive breast cancers into prognostic categories and therefore may also be useful for therapeutic decisions.

The study was performed by immunohistochemistry for Her2 receptor and Htf9c on (paraffin embedded) tissue microarray (TMA) assembled from 3 different institutions with a total number of 214 Her2 positive breast cancers. The relevance of Htf9c was proved by uni- and bivariate Hazard ratios.

The issue addressed by the authors is of wide clinical interest and supposed to affect daily work of pathologists and oncologists in case of clinical establishment.

Several expression assays have been developed and markers have been published over the past several years to distinguish invasive breast cancers superiorily than the established clinicopathologic parameters regarding prognosis and prediction. So far, only Agendia-MammoprintTM has been FDA approved.

Less is known from the cell-cycle dependent regulated protein Htf9c. The role of Htf9c in tumorigenesis and –progression or proliferation is unclear. This study displays a descriptive and statistical correlation of Htf9c with outcome without targeting biologic causality.

**Major concerns:**

1. What is the specific immunhistochemical staining of Htf9c? The authors have regarded cytoplasmic staining as specific, indeed Htf9c is an cell cycle regulated protein.

2. Determination of the Her2 status is done by immunohistochemistry (HerceptestTM). More accurate -because of better prediction of response to therapy with TrastuzumabTM- is its determination by FISH analysis.

3. TMA from 2 of the 3 cohorts used in this study were also used in the study of Ring et al. (JCO 2006). Ring et al. have considered tumors as Her2 positive, when they have been scored 2+ or 3+ (table 1). This needs further explanation, then score 2+ in immunohistochemistry means equivocal status and should subsequently tested by FISH. Was Her2 status determined according to the ASCO 2007 guideline?

4. In the publication Ring et al. the total number of Her2 positive tumors was declared with 68 and 34 for the CCIH and CCF cohort, respectively. Using the same TMA for this study, the total number of Her2 positive tumors for the CCH cohort is declared with 81. The difference of 47 has to be explained.
5. In the publication of Ring et al. the univariate association with outcome shows a weak negative correlation with Htf9c in the CCIH and CCF cohort but a positive correlation with the BBCA cohort. Interestingly, in this study the BBCA cohort was replaced by the RPCI cohort.

As the authors discussed, it should be noted, that these cohorts were not derived from randomized trials. The study is restricted in his power due to the methods and the investigated cohorts. The cohorts are too small and too heterogeneous especially because of different treatment of the patients. This is also reflected by the wide range of Htf9c positivity in the different cohorts (29%, 37% and 23%) and by the bivariate Hazard ratios (e.g. 12.25, 2.79 and 3.34 for grade and Htf9c).

Title and abstract accurately convey the entire content of the work. It is written comprehensible. In the abstract TrastuzumabTM is misspelled.

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

Statistics could be reviewed by Inti Zlobec (Institute of Pathology, University Hospital Basel, Switzerland, IZlobec@uhbs.ch).

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