Author's response to reviews

Title: Expression of aurora kinase A is associated with metastasis-free survival in node-negative breast cancer patients

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Author's response to reviews: see over
Reviewer 1

Reviewer’s comment: “ER and HER2 status was derived from the gene array data. ER and HER2 status should be assessed by using standard methods (i.e. IHC and in situ hybridization, respectively) in order to verify the major finding in a clinical setting. Alternatively, the authors could at least show how differently assessed ER and HER2 statuses correlate (standard methods vs gene array data). Authors state that ER, PR and HER2 status were derived from RNA levels as described in Schmidt et al., 2010. The cited study is entitled “Ep-CAM RNA expression predicts metastasis-free survival in three cohorts of untreated node-negative breast cancer”. No detailed description of how ER, PR and HER2 status were derived from gene array data was found in that article.”

Response: This is an important aspect. In the Mainz cohort, both ER and HER2 immunostaining as well as RNA analyses (gene array) was available. Both ER (ESR1) and HER2 (ERBB2) RNA levels showed a bimodal distribution.

Using a statistical procedure that determines the nadir of frequency distribution curves we differentiated between patients with low and high ESR1 and ERBB2, respectively, based on RNA levels. The dichotomization based on RNA levels is in very good agreement with ER and HER2 status assessed by standard immunostaining (table below). Because of the good correlation we think it is justified to use the RNA data to differentiate between ER as well as HER2 high and low expressing carcinomas. It should also be considered that for some breast cancer cohorts with available gene array data ER and HER2 IHC or in situ hybridization data have not been published. This information was added to materials and methods and to the supplemental.

Reviewer’s comment: “It could also be noted that there was a discrepancy in the Mainz cohort numbers: in 2010 the Mainz cohort (n=194) included 149 ER-positive patients and now authors report 169 ER-positive patients of 200. Even if the total number of Mainz cohort is slightly different (difference of 6 patients), the assessment of ER status could not have been done based on the same criteria. The definition criterion of the subgroups is here of great importance, since the major result of the current study was derived from the multivariate analysis of the ER+/HER2-subgroup.”

Response: This is correct and the six carcinomas mentioned by the reviewer have already been intensively discussed. They represent DCIS with microinvasion. In our original publication where we introduced the Mainz cohort we included all 200 patients of node negative breast cancer including these 6 microinvasive cases (Schmidt et al., 2008). However, upon a reevaluation our pathologists recommended to treat the six patients with care, because they were classified microinvasive only. This is the reason why they were not included into Schmidt et al., 2010. However, the significance to the statistical analyses in Schmidt et al. (2010) does not depend on whether the patients are included or not. Later upon careful reanalysis of these six “difficult” patients it became clear that it is indeed justified to consider them as invasive carcinomas. Therefore, we included them again into the current analysis. However, it should be considered that all results reported as statistically significant in the current manuscript would also be significant after omission of the six patients. In
conclusion, the result in this and in our previous study is similar whether the six patients are included or not.

Reviewer’s comment: “Authors have previously published identical AURKA expression study innode-negative breast cancer patients (reference number 18, Cancer Res 2010; 70(24 Suppl):Abstract nr P3-10-22). Authors could clarify why they ended up with different statistics with the same data set of 766 patients. In 2010 they report association of high AURKA expression with metastasis-free survival in univariate analysis of the whole patient cohort (HR 1.523, 95% CI 1.343-1.726, P<0.001). In the current study authors report HR 1.669, 95% CI 1.402-1.086, P<0.001.”

Response: Indeed, in a poster abstract (for the San Antonio meeting) published 2010 we reported an association of high AURKA expression with metastasis-free survival in univariate analysis of the whole patient cohort (HR 1.523, 95% CI 1.343-1.726, P<0.001) (Cancer Res 2010; 70(24 Suppl):Abstract nr P3-10-22). In the current manuscript the values are: HR 1.669, 95% CI 1.402-1.086, P<0.001. The reason for the discrepancy is that for the 2010 abstract (and the papers before) we used MAS5 for normalization of the gene array data. Later, RMA became the method of first choice which we also used in the current manuscript. Please consider that the discrepancy is not large and that convincing P-values (<0.001) were obtained for both normalization techniques. Please consider that also in the revised version some numerical changes occur in the multivariate analysis. The reason is that we could obtain an updated version with information on grading that was missing for some patients in the previous version.

Reviewer’s comment: “Why multivariate Cox analysis included only Mainz and Transbig cohorts? In the multivariate subgroup analyses there are nevertheless patients included from all the 3 cohorts.”

Response: Unfortunately, no information on grading and stage has been published for the Rotterdam cohort. Therefore, these patients could not be included into the analysis.

Reviewer’s comment: “AURKA IHC would strengthen the impact of the study.”

Response: We agree, but paraffin sides are only available to us for a fraction of the Mainz cohort. Unfortunately, our resources are currently not sufficient to perform this additional study.

Reviewer’s comment: “Authors have reported significant association of Ep-CAM expression with metastasis-free survival in the same data set: could Ep-CAM expression be included in the multivariate analyses?”

Response: This is an important suggestion. The likelihood ratio statistic shows that AURKA adds independent prognostic information over Ep-CAM in the cohort of all patients as well as in the ER+/HER2- subgroup (Supplemental Fig. 1).

Reviewer’s comment: “Minor Essential Revisions:
1. First line in the abstract: the word ‘kinase’ is repeated unnecessarily.
2. Ninth line of discussion: AURKA is spelled AUKA.”
Discretionary Revisions:
1. *Tables describing the clinicopathological characteristics of three cohorts could be united.*

Response: We deleted “kinase” and corrected “AUKA”. However, we would prefer not to unite the tables with clinicopathological characteristics because we show the prognostic influence in each of these three independent datasets.
Reviewer 2

Reviewer’s comment: “This is a statistically sound, well-written study of the prognostic value of aurora kinase in several medium-sized cohorts of ER+ breast cancer patients, as assessed by RNA expression microarray. It essentially confirms previous findings regarding the prognostic value of Aurora kinase in ER+ breast tumours.

Minor essential revisions: The recent study by Ali et al (Br J Ca 2012, "Aurora kinase A outperforms Ki67 as a prognostic marker in ER-positive breast cancer") ought to be cited, as it recently made similar findings in a large tumour cohort, by Immunohistochemistry."

Response: We thank the reviewer for this important suggestion. The study of Ali et al. was included into the manuscript.

“Meanwhile, in a head to head comparison of a large panel of proliferation markers using immunohistochemistry in 3.093 breast carcinomas AURKA outperformed other proliferation markers as an independent predictor of breast cancer-specific survival in ER-positive breast cancer [1].”

Reviewer 3

Reviewer’s comment: “Page 6: AURKA has been identified as a good predictor of survival in breast cancer in numerous studies (see references below). Description of these papers is insufficient and the introduction should be updated to reflect their findings.

Refs:
- and references 24 and 27 in the text.

Actually AURKA has also been selected in at least 182 microarray gene signatures as reported in GeneSigDB (http://compbio.dfci.harvard.edu/genesigdb); among these signatures, one can cite the extended mammaprint signature (231 genes) published by van’t Veer et al., the Gene expression Grade Index (GGI) published by Sotiriou et al., and the 72 proliferation genes published by Reyal et al.”

Response: We thank the reviewer for this important suggestion and included all references into the manuscript:

“Gene expression profiling has led to a magnitude of different signatures which a related to breast cancer prognosis. In a meta-analysis of publicly available breast cancer gene
expression and clinical data, Wiripati and co-workers underscored the important role of proliferation in breast cancer prognosis [2]. Clearly, there are numerous proliferation-associated genes. Martin and co-workers used a novel unsupervised approach to identify a set of genes whose expression predicts prognosis of breast cancer patients [3]. Amongst the most predictive genes for ER positive patients was AURKA, a gene which is a constituent in multiple microarray gene signatures [4–6].

Meanwhile, in a head to head comparison of a large panel of proliferation markers using immunohistochemistry in 3,093 breast carcinomas AURKA outperformed other proliferation markers as an independent predictor of breast cancer-specific survival in ER-positive breast cancer [1]. Finally, a sophisticated analysis of prognostication strategies in breast cancer microarray data sets showed that that the most complex methods were not necessarily better than a univariate model relying on a single gene like AURKA [7].

However, we would prefer to leave the references to Desmedt et al., 2008 and Haibe-Kains et al., 2012 in the discussion section of our manuscript because we feel that otherwise a strong predominance of the introduction section would occur.

Reviewer’s comment: “Page 6: The authors wrote "seemingly contradictory results on the role of AURKA in breast cancer prognosis". There is plenty of evidence that AURKA expression is highly prognostic and is negatively correlated with survival in ER+ breast cancer patients. Why do the authors think the published results are contradictory?
Page 11: If the authors refer to ref 16 as the discrepancy, it is only one paper among the plethora of publications consistently identifying AURKA as a strong prognostic factor."

Response: Yes, the “discrepancy” in our first version of the manuscript was based on ref 16. However, we agree to the reviewer that this seems problematic when several studies found a clear difference. We removed this sentence.

Reviewer’s comment: “Page 10: Given the high correlation of AURKA and histological grade it is unclear whether there is a real benefit of considering AURKA expression in addition to the traditional clinical parameters. I would suggest the authors to perform an analysis similar to Figure 1 of the last paper of Prat et al. - Prat A et al.: PAM50 assay and the three-gene model for identifying the major and clinically relevant molecular subtypes of breast cancer. Breast Cancer Res. Treat. 2012.
Minor Essential Revisions“

Response: This is a very helpful suggestion. We added the additional analysis by including an additional figure (Fig. 5 and supplemental Fig. 1):

Results: “Given the high correlation of AURKA and histological grade and the association of grading with prognosis we analyzed whether there is a real benefit of considering AURKA expression. For this purpose we performed an analysis similarly as Prat et al.(2012). To compare the amount of independent prognostic information
provided by AURKA we estimated the likelihood ratio statistic in a model that already included grading (Fig. 5). The model showed that AURKA provided significant additional information over grading in the cohort of all patients, as well as in the ER+/HER2- and in the HER2+ subgroups. In a previous publication Ep-CAM was described as strong prognostic factor in breast cancer [8,9]. The likelihood ratio statistic shows that AURKA also adds independent prognostic information over Ep-CAM in the cohort of all patients as well as in the ER+/HER2- subgroup (Supplemental Fig. 1).

Reviewer’s comment: “Page 7: Because the gene expression data come from three different patients cohorts and datasets, one should check for batch effect. Batch effect correction could be performed if necessary. Frozen RMA could also be used in place of RMA to reduce batch effects.”

Response: We agree. Meanwhile we re-normalized the gene array data by frozen RMA as suggested by the reviewer. It is out of question that there are clear batch effects between the three subcohorts. However, since AURKA is significantly associated with prognosis in all three subcohorts, the effect reported in this manuscript is certainly not an artifact due to batch effects.
References


