Author's response to reviews

Title: In situ aromatase expression in primary tumor is associated with estrogen receptor expression but is not predictive of response to endocrine therapy in advanced breast cancer

Authors:

Anne E Lykkesfeldt (al@cancer.dk)
Katrine L Henriksen (khe@cancer.dk)
Birgitte B Rasmussen (bibbra01@heh.regionh.dk)
Hironobu Sasano (hsasano@patholo2.med.tohoku.ac.jp)
Dean B Evans (dean.evans@novartis.com)
Susanne Møller (sm@dbcg.dk)
Bent Ejlertsen (bent.ejlertsen@rh.regionh.dk)
Henning T Mouridsen (mou@dbcg.dk)

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Author's response to reviews:

BMC Cancer
Covering letter

Dear Editor,

Please receive this revised manuscript “In situ aromatase expression in primary tumor is associated with ER expression but is not predictive of response to endocrine therapy in advanced breast cancer”, for publication in BMC cancer.

Regarding the comments from reviewers they are addressed point-by-point below.

Reviewer 1:
1) Discussion of our results in relation to other publications has been added to the discussion (p.18, 19 and 20).

2) Regarding discrepancy between aromatase measurements in whole sections and tissue microarrays: We have included pictures of IHC aromatase staining of both WS (Fig 1E) and TMA (Fig 1F) illustrating the heterogeneous distribution of intratumoral aromatase which we believe is the cause of discordant results between WS and TMA measurements. In contrast to aromatase, no discordance was found for ER, PR and COX-2 as reported in KL Henriksen et al. J Clin Pathol 2007,60:397-404. This reference is included in the reference list.

Reviewer 2:
1) We have included a new figure (Figure 1) of IHC aromatase staining from both WS and TMAs, illustrating both high and low level of aromatase expression and
examples from tumors with discordant results in WS and TMA staining.

2) We have suggested that our data indicate that “local estrogen synthesis is not the major source of intratumoral estrogen”, because we agree with the reviewers that we have only circumstantial data. We have now rephrased the sentence to ensure that it appears as a suggestion.

3) The mechanisms of aromatase induction have been added to the introduction.

4) The importance of ER as prognostic factor is now mentioned in the introduction. It is correct that ER negative tumors may express PR, but the fraction of ER-/PR+ in postmenopausal patients is low (around 3% in Danish patients (SM Thorpe, Acta Oncol 1988, 27:1-19) and in this study only 5 ER-/PR+ tumors were included, excluding the possibility of correlation analysis. Thus, we find it meaningful to look at aromatase expression in relation to high PR expression level.

5) To shorten the paper we have removed the supplementary data which can be found in Table 1 and 2 in KL Henriksen et al. Acta Oncl. 2009 48:225-36. Sections in the discussion have also been revised and shortened.

Reviewer 4:

1) Due to the knowledge of aromatase expression in both fibroblasts, adipose cells, benign duct cells and invasive cancer cells, we originally scored aromatase expression in all four compartments. However, since only the carcinoma cell staining with the #667 antibody was correlated with the aromatase enzyme activity (H Sasano et al., 2005), we have focused on the carcinoma cells and the correlation analyses have only been performed between carcinoma cells. To be more specific in the revised manuscript, we have now included “carcinoma cells” at all relevant places.

2) The reference ”KL Henriksen et al. J Clin Pathol 2007,60:397-404” has been included.

3) We did not find correlation between ER and PR expression, and it was not a surprising result for us. The ER protein has reduced stability upon binding of ligand (M Borrás et al. J Steroid Biochem Mol Endocrinol 1994 48:225-36). Therefore, a low ER expression level may be due to low expression of un-liganded ER and consequently no PR induction. However, it could also be due to low ER level resulting from turn-over of ligand-bound ER, which is expected to be associated with high PR expression. Similarly, moderate level of ER may be associated with high PR expression, but may also reflect tumors with moderate level of free ER (un-liganded ER), which do not express PR. Accordingly, high ER level may be associated with both low and high PR expression.

4) The section in the discussion regarding COX-2 as a surrogate marker for aromatase expression has been revised. Comments on epithelial-stromal interactions have also been added to the discussion.
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5) We have read the suggested paper by DB Hardy et al. 2008 Mol. Endocrinol. 22:1812-1824 and it has been included as reference in the discussion.

We hope that you can accept our revised manuscript for publication in BMC cancer.

Yours sincerely,

Anne E. Lykkesfeldt

Head, Dept. Tumor Endocrinology

Institute of Cancer Biology

Danish Cancer Society

DK- 2100 Copenhagen Ø, Denmark

Tel: +45 3525 7323

Fax: +45 3525 7721

E-mail: al@cancer.dk