Adjuvant sequencing of tamoxifen and anastrozole is superior to tamoxifen alone in postmenopausal women with low proliferating breast cancer

Zsuzsanna Bago-Horvath¹,⁵, Margaretha Rudas¹,⁵, Peter Dubsky²,⁵, Raimund Jakesz²,⁵, Christian F. Singer³,⁵, Ralf Kemmerling⁶, Richard Greil⁷, Andrea Jelen⁸, Gerhard Böhm⁹, Zerina Jasarevic¹⁰, Anton Haid¹¹, Christine Gruber¹², Sabine Pöstlberger¹³, Martin Filipits⁴,⁵, Michael Gnant²,⁵ for the Austrian Breast and Colorectal Cancer Study Group*

¹Departments of Pathology, ²Surgery, ³Special Gynecology, and ⁴Medicine I, ⁵Comprehensive Cancer Center, Medical University of Vienna, Vienna; ⁶Departments of Pathology and ⁷Internal Medicine III, General Hospital Salzburg, Paracelsus Private Medical University, Salzburg; ⁸Department of Pathology and Microbiology, Hanusch Medical Center, Vienna; ⁹Department of Pathology, Hospital Oberwart, Oberwart; ¹⁰Department of Pathology and ¹¹Department of Surgery, Academic Teaching Hospital Feldkirch, Feldkirch; ¹²Department of Pathology and ¹³Surgery, Hospital of the Sisters of Mercy, Linz – all in Austria.

Correspondence to: Martin Filipits, PhD; Associate Professor
Institute of Cancer Research, Department of Medicine I
Comprehensive Cancer Center, Medical University of Vienna
Borschkegasse 8a, A-1090 Vienna, Austria
Phone: +43 1 4277 65237; Fax: +43 1 4277 65192
E-mail: martin.filipits@meduniwien.ac.at

Running head: Ki67 in breast cancer
*Other investigators and team members of the Austrian Breast & Colorectal Cancer Study Group (ABCSG) who contributed in the study are listed in the Appendix.

**Statement of Clinical Relevance**

Ki67 is an important prognostic factor and may also be a predictive biomarker in breast cancer. In the present study, we determined Ki67 expression centrally by immunohistochemistry on whole tissue sections of postmenopausal endocrine-responsive breast cancers from patients who had been enrolled in the prospectively randomized ABCSG Trial 8, and had received tamoxifen (TAM) for 5 years, or TAM for 2 years followed by anastrozole (ANA) for 3 years. Our findings suggest that adjuvant sequencing of TAM and ANA is particularly beneficial in lowest risk patients with medium or high ER, low proliferating breast cancer. If these results are confirmed, this would suggest that estrogen depletion via aromatase inhibition is a more effective treatment in breast cancers with high dependence on estrogen metabolites.
Abstract

**Purpose:** To assess the predictive value of Ki67 expression in postmenopausal hormone receptor-positive early breast cancer patients who were either treated with adjuvant tamoxifen (TAM) alone or with TAM followed by anastrozole (ANA).

**Experimental Design:** Expression of Ki67 was determined centrally by immunohistochemistry on whole tissue sections of postmenopausal endocrine-responsive breast cancers from patients who had been enrolled in the prospectively randomized Austrian Breast and Colorectal Cancer Study Group (ABCSG) Trial 8, and had received TAM for 5 years, or TAM for 2 years followed by ANA for 3 years. Ki67 expression was evaluated both as a continuous variable and dichotomized to low (≤10%) and high (>10%). Recurrence-free survival (RFS) and overall survival (OS) were analyzed using Cox models adjusted for clinical and pathological parameters.

**Results:** Patients with a high Ki67 expression (394/1,587; 23%) had a significantly shorter RFS (adjusted hazard ratio [HR] for recurrence 1.90, 95% confidence interval [CI] 1.37-2.64, p=0.0001) and OS (adjusted HR for death 1.78, 95% CI 1.18-2.70, p=0.006). In women with breast tumors expressing medium or high ER levels (n=1,438), the interaction between Ki67 and adjuvant endocrine treatment was significant for RFS (p=0.03). TAM followed by ANA was superior to TAM alone in patients with low Ki67 (adjusted HR 0.53, 95% CI 0.34-0.83, p=0.005) but not in high Ki67 disease (adjusted HR 1.18, 95% CI 0.66-1.89, p=0.68).

**Conclusions:** Adjuvant sequencing of TAM and ANA is superior to TAM alone particularly in postmenopausal women with medium or high ER expressing, low proliferating breast cancer.
Introduction

Assessment of tumor proliferation plays a central role in the stratification of breast cancer into biologically relevant, distinct subtypes. One of the most feasible and direct ways to measure tumor proliferation is the immunohistochemical determination of Ki67 expression. Ki67, a proliferation marker, is present solely in cycling cells. In recent years, this marker has been drawn into the spotlight of interest as a prognostic factor and predictor of therapy response in early breast cancer.(1-9) Several studies have demonstrated that in hormone receptor-positive, early breast cancer, Ki67 serves as a prognostic factor in both pre- and postmenopausal women. In addition, high expression of Ki67 predicts response to chemotherapy in the neoadjuvant setting, and dynamic changes in Ki67 expression have also been found to correlate with response to endocrine therapy with aromatase inhibitors.(5, 10-12)

Ki67 is a crucial factor that distinguishes luminal A from luminal B breast cancers with a simple methodology. Luminal A cancers, which bear a better prognosis, possess a lower fraction of Ki67-expressing tumor cells, in contrast to the luminal B subtype with a higher fraction of cycling cells, which indicated worse prognosis.(8) However, the optimal therapeutic approaches in the adjuvant treatment of luminal B breast cancer remain to be determined. One central question is whether these patients benefit from endocrine treatment with aromatase inhibitors over tamoxifen (TAM).

It has been reported that high Ki67 labeling index might be predictive of benefit from letrozole over TAM in postmenopausal patients with hormone receptor-positive breast cancer.(7) In the present study, we investigated the clinical relevance of immunohistochemically determined Ki67 expression in terms of recurrence-free survival (RFS) and overall survival (OS) in postmenopausal women with estrogen receptor (ER)-positive breast cancer and limited risk who were enrolled in the Austrian Breast and
Colorectal Cancer Study Group (ABCSG)-8 trial and were prospectively randomized to 5 years of adjuvant TAM or 3 years of anastrozole (ANA) after 2 years of TAM, in the absence of adjuvant chemotherapy. We hypothesized that sequencing of TAM and ANA may have differential impact on RFS and OS in patients with low and high proliferating breast cancers, respectively.

**Patients and Methods**

**Patients**

The present investigation is part of the ABCSG translational research program (abcsg.research). All patients included in this study had participated in the large phase III trial ABCSG-8. The study design, inclusion criteria and the main results of the trial have been reported elsewhere.(13) The objective of ABCSG-8 was to determine whether the sequence strategy of TAM followed by ANA improves the outcome of postmenopausal women with hormone receptor-positive, early-stage breast cancer. Briefly, a total of 3,714 postmenopausal breast cancer patients had been randomly assigned to receive either TAM for 5 years or TAM for 2 years followed by ANA for 3 years. Patients included in ABCSG-8 did not receive neo-adjuvant or adjuvant chemotherapy as well as trastuzumab. The primary endpoint was RFS defined as time from randomization to the earliest occurrence of local or distant recurrence or death from any cause.

The sequence strategy of 2 years of TAM followed by 3 years of ANA leads to moderate outcome benefits and lends further support to the inclusion of ANA during the first five years of endocrine therapy.
Specimen collection and immunostaining for Ki67

A block containing representative formalin-fixed, paraffin-embedded tumor tissue was available from 1,587 out of 3,714 (43%) patients enrolled into ABCSG-8 and sent to the central lab of abcsg.research at the Medical University of Vienna. A flowchart illustrating patient and sample selection is shown in Figure 1. In total, 19 centers contributed tumor samples (see appendix). All tumor specimens were obtained at the time of surgery before adjuvant therapy. Paraffin blocks were stored at room temperature and were identifiable only by an identification number assigned to each patient at randomization. Approval was obtained from the local institutional review boards. From each tumor block sections were cut at 4 µm. One section was stained by hematoxilin and eosin to confirm the presence of invasive carcinoma histologically and further sections were used for immunohistochemical analyses as described previously.

Immunohistochemistry was performed and evaluated centrally in the abcsg.research central lab at the Medical University of Vienna by using a standardized protocol: Briefly, tissue sections were deparaffinized and rehydrated. After heating for 10 minutes in 10 mM citrate buffer (pH 6.0) in a pressure cooker for epitope retrieval, the tissue sections were incubated for 30 minutes at room temperature with a mouse monoclonal antibody specific for Ki67 (clone MIB1, Dako, dilution 1:100). Antibody binding was detected by means of the UltraVision LP detection system according to the manufacturer’s recommendations (Lab Vision Corporation). Color development was performed by 3-3’-diaminobenzidine and counterstaining by hematoxylin. HER2 status was centrally assessed by immunohistochemistry. In case of HER2 2+ scores, FISH analyses were used to determine amplification.

Ki67 and HER2 immunostaining was independently assessed by experienced breast pathologists (Z.B.-H., M.R.), who were unaware of the patients’ clinical data. For Ki67, invasive tumor cells in 20 representative high power fields (HPF, 400 x magnification)
were visually evaluated and only nuclear staining was scored as positive. The results were documented as the percentage of Ki67-stained nuclei regardless of staining intensity. Comparisons of Ki67 expression with clinical variables including survival of the patients were done with Ki67 expression as a continuous variable and as a dichotomized variable classified as low (≤10%) and high (>10%). This cut-off of 10% was previously used in several series published by others.(1, 14)

**Statistical analyses**

The primary endpoint of this analysis was RFS defined as time from randomization to the first occurrence of local, regional or distant recurrence or death from any cause, as in the ABCSG-8 trial. OS defined as time from randomization to death from any cause was a secondary endpoint.

In order to be consistent with the clinical trial data presentation and to maintain consistency, we censored all patients at the time when they were allowed to enter ABCSG-16 (Secondary Adjuvant Long Term Study With Arimidex; SALSA, NCT00295620) or at 5 years. In addition, patients who selectively crossed over to the sequential treatment arm were censored at the time of cross-over (Dubsky et al., manuscript submitted for publication).

Baseline characteristics of patients with or without tumor blocks were compared using the chi-square test. Baseline data according to dichotomized Ki67 status (≤10%, >10%) were compared in univariate analyses using the chi-square test and in a multiple logistic model. Survival rates were estimated with the use of the Kaplan–Meier method. The prognostic value of breast cancer subtypes was studied using univariate and multiple Cox models. All multiple regression models (i.e. logistic and Cox) mentioned below were adjusted for age, tumor size, lymph node status, tumor grade, ER expression, progesterone receptor (PgR) expression, HER2, and adjuvant endocrine treatment. All
reported P-values are results of two-sided tests. A P-value equal to or less than 5% was considered statistically significant. All statistical analyses were performed using of SPSS software, version 15.0 (SPSS Inc.).

Results

Ki67 and patient characteristics

We centrally assessed immunostaining of Ki67 on whole tumor tissue sections of 1,587 patients. As shown in Table 1, these 1,587 patients had similar pre-randomization characteristics as the clinical trial. Of the 1,587 tumors, 394 (23%) had a high (>10%) Ki67 expression. The median numeric value of Ki67 expression was 10% in all 1,587 patients and also 10% in both treatment arms of the clinical trial. The association of Ki67 with clinical variables is shown in Table 1. High Ki67 was associated with larger tumors, higher tumor grade, lower PgR expression levels, and HER2 positivity in univariate analyses. In addition, we compared baseline data according to Ki67 with a multivariate logistic model. Ki67 positivity was studied simultaneously according to age, tumor size, nodal status, tumor grade, ER expression, PgR expression, HER2 expression, and adjuvant endocrine treatment. In these analyses, high Ki67 remained associated with tumor size (odds ratio [OR] 1.35, 95% confidence interval [CI] 1.06-1.72; p=0.01), tumor grade (OR 1.32, 95% CI 1.12-1.54; p=0.001), PgR expression (OR 0.84, 95% CI 0.75-0.94; p=0.002), and HER2 expression (OR 2.75, 95% CI 1.80-4.20; p<0.0001).

Prognostic Analysis

As in the ABCSG-8 trial, all patients were censored at 60 months follow-up irrespective of eventual extended adjuvant treatment. Therefore, the median follow-up in this
analysis is 60 months. Within the first 5 years of follow-up, 157 of 1,587 (9.9%) patients had local, regional or distant recurrences, 98 of 1,587 (6.2%) patients had died. The 5-year RFS and OS rates were 89.5% and 93.5% in this study population, respectively. RFS and OS were significantly shorter for patients with tumors with high Ki67 (hazard ratio [HR] for recurrence 2.28, 95% CI 1.66-3.13, p<0.0001; HR for death 2.15, 95% CI 1.44-3.22, p=0.0002), confirming the prognostic value of Ki67 in this cohort (Figure 2A and 2B). Five-year RFS and OS estimates were 92% and 95% for low versus 83% and 89% for high Ki67 labeling index, respectively. In addition to Ki67, age, tumor size, nodal status and PgR expression were also significantly associated with RFS in the univariate analyses (Table 2). Age, tumor size, and nodal status were associated with OS as well. The Cox models were adjusted for these variables plus tumor grade, ER expression and HER2 expression. In these multivariate analyses, Ki67 was still significantly associated with RFS (adjusted HR for recurrence 1.90, 95% CI 1.37-2.64, p<0.0001) and OS (adjusted HR for death 1.78, 95% CI 1.18-2.70, p=0.006) of the patients (Table 2). Similar results were obtained when Ki67 was included as continuous variable into the Cox model (adjusted unit HR for recurrence 1.03, 95% CI 1.02-1.04, p<0.0001; adjusted unit HR for death 1.02, 95% CI 1.01-1.04, p=0.007). Thus, Ki67 is an independent poor prognostic factor.

**Predictive Analysis**

The interaction of Ki67 and treatment benefit was evaluated in a Cox model adjusted for age, tumor size, nodal status, tumor grade, ER expression, PgR expression, and HER2 in the total patient population and in women with medium or high ER expressing breast cancer. In the subgroup analysis of patients with medium or high ER tumors, ER was not included into the Cox model. In all 1,587 patients, the test for interaction between Ki67 and treatment on RFS was marginally significant (p=0.049) but it was not
significant for OS (p=0.79). These data suggest that adjuvant sequencing of TAM and ANA leads to a significantly prolonged RFS compared to TAM alone in postmenopausal women with low Ki67 (adjusted HR for recurrence 0.61, 95% CI 0.40-0.92, p=0.02) but not in high Ki67 disease (adjusted HR for recurrence 1.18, 95% CI 0.72-1.93, p=0.52). This treatment-by-marker effect was even more pronounced in the 1,438 women with medium or high ER breast cancer (test for interaction p=0.03). TAM followed by ANA was superior to TAM alone in postmenopausal women with medium or high ER tumors and low Ki67 (adjusted HR for recurrence 0.53, 95% CI 0.34-0.83, p=0.005) but not in high Ki67 disease (adjusted HR for recurrence 1.18, 95% CI 0.66-1.89, p=0.68) (Figure 3). RFS at 5 years was 89% (TAM) and 94% (TAM→ANA) for low and 83% (TAM and TAM→ANA) for high Ki67 labeling index, respectively (Figure 3).

**Discussion**

In this study, we confirmed that high Ki67 is associated with both shorter RFS and OS in early-stage breast cancer. Thus, high Ki67 is an independent poor prognostic factor in women with breast cancer who received endocrine therapy which has been previously described.(5, 7, 9) Breast carcinomas with high Ki67 expression are most likely luminal B type cancers and may have, in addition to estrogen-driven proliferation, estrogen-independent pathways activated. This might be one of the reasons why some of these tumors are intrinsically resistant to endocrine therapy.(8) Moreover, we observed a significant treatment-by-marker effect. Therefore, Ki67 is also a predictive marker in ABCSG-8 patients, suggesting that adjuvant sequencing of TAM and ANA may be superior to TAM alone particularly in postmenopausal women with medium or high ER, low proliferating breast cancer.
The BIG 1-98 findings suggested that Ki67 may have predictive value among
postmenopausal women with endocrine responsive early breast cancer.(7) Comparison
of letrozole and TAM by Ki67 revealed that letrozole may be particularly beneficial at
higher levels of Ki67 which is in contrast to our results.

There may be several reasons behind this apparent difference. The positive interaction
between high proliferation and letrozole in BIG 1-98 was described in a monotherapy
setting (letrozole for 5 years versus TAM for 5 years), whereas ABCSG-8 tested the
interaction in a sequence of anastrozole after two years of TAM. Furthermore, the
benefit of anastrozole is carried over the 60 months of endocrine treatment. A recent
update of the ATAC trial clearly indicates that the reductions in risk are sustained well
beyond the five years of treatment.(15) Our analysis does not account for recurrences
after five years and merely represents the early outcome of these women.

A comparison of characteristics of patients from ABCSG-8 with BIG 1-98 for whom Ki67
was available showed that 72% of ABCSG-8 patients were node negative and 72% had
tumors below 2 cm. In the BIG 1-98 subpopulation a rate of 65% node negative patients
and 65% of tumors below 2 cm was observed. Furthermore, ABCSG-8 was the only
large endocrine treatment trial that excluded G3 tumors from the trial population,
whereas in BIG 1-98 16% of patients had G3 tumors. Another important difference may
be that ABCSG-8 did not allow chemotherapy, whereas in BIG 1-98 26% of patients
received preoperative or adjuvant chemotherapy. Although the Cox proportional-
hazards regression analysis in BIG 1-98 was stratified according to chemotherapy use,
this could be of importance because it has been shown that high Ki67 is associated with
a better outcome of chemotherapy thus suggesting that patients with highly proliferating
tumors may be undertreated with the adjuvant regimens used in ABCSG-8.(10-12)

The median value of 10% for Ki67 in ABCSG-8 is comparable to the median of 11% in
BIG 1-98 and similar to the 10% cut off used in other studies.(1, 14) In both trials,
ABCSG-8 and BIG 1-98, a correlation between high Ki67 and adverse prognostic factors such as larger tumor size, positive nodal status, higher tumor grade and HER2 positivity was observed.

The prognostic and predictive value of Ki67 was also assessed in two randomized trials of the International Breast Cancer Study Group (IBCSG Trials VIII and IX) that compared adjuvant chemo-endocrine therapy with endocrine therapy alone in patients with node-negative breast cancer.(9) In postmenopausal women, a high Ki67 was associated with other factors that predict poor prognosis such as larger tumor size, higher tumor grade, and the absence of ER and PgR expression and HER2 overexpression. Among patients with endocrine-responsive tumors, a high Ki67 was associated with worse disease-free survival but the Ki67 did not predict the relative efficacy of chemo-endocrine therapy compared with endocrine therapy alone. Thus, Ki67 was an independent prognostic factor but was not predictive of better response to adjuvant chemotherapy in these studies.

The present findings suggest that adjuvant sequencing of TAM and ANA is particularly beneficial in lowest risk patients with a low Ki67 labeling index. If these results are confirmed by other translational studies from adjuvant endocrine or neo-endocrine trials this would suggest that estrogen depletion via aromatase inhibition is a more effective treatment in breast cancers with high dependence on estrogen metabolites. This is also supported by recent findings of the Tamoxifen and Exemestane Adjuvant Multinational (TEAM) pathology substudy, which showed a trend toward benefit in favor of exemestane compared to tamoxifen in patients with breast tumors expressing high ER levels.(16) In patients with higher proliferation (associated with larger tumor size, higher tumor grade and lower ER expression), the type of endocrine treatment may be less relevant.
However, the optimal adjuvant therapy in this patient population with higher risk remains currently unknown and has to be determined in well-designed prospective clinical trials. Thus if our results are confirmed, Ki67 may be another useful biomarker to identify postmenopausal women with endocrine responsive early breast cancer with low risk. In addition this population may have particular benefit from adjuvant sequencing of TAM and ANA. Finally, an individual patient level pooling project adding data from all aromatase inhibitor translational studies may have sufficient power and diversity of subjects to build and validate a really persuasive predictive model.

Appendix

The following investigators also participated in this study:

T. Bachleitner-Hofmann, S. Taucher, U. Pluschnig, R. Bartsch, G. Steger, C. Zielinski, S. Horvath, M. Seifert, C. Dadak, R. Obwegeser, E. Kubista, E. Asseryanis, R. Möslinger-Gehmayer, E. Hanzal, C. Sam (Medical University of Vienna, Vienna); P. Mayer, C. Menzel, B. Mlineritsch, M. Moik, C. Rass, R. Reitsamer, G. Russ (Salzburg Hospital, Salzburg); K. Haider, W. Kwasny, E. Markis, W. Stiglbauer (Wiener Neustadt Hospital, Wiener Neustadt); R. Grill, M. Medl, A. Nader, M. Stierer (Hanusch Hospital, Vienna); S. Redtenbacher, W. Ulrich (Hietzing Hospital, Vienna); G. Reiner (Weinviertel Hospital, Mistelbach); F. Hofbauer, M. Lang (Oberpullendorf Hospital, Oberpullendorf); W. Horvath (Güssing Hospital, Güssing); P. Kier, A. Reiner-Concin, K. Renner (Sozialmedizinisches Zentrum Ost Hospital, Vienna); P. Balcke, G. Salem, R. Sedivy (St. Pölten Hospital, St. Pölten); E. Nessler, W. Neunteufel (Dornbirn Hospital, Dornbirn); A. Haid, F. Offner (Academic Teaching Hospital Feldkirch, Feldkirch); F. Kugler, G. Michlmayer, R. Schildberger, W. Sega (Sisters of Mercy Hospital, Linz); F. Fridrik, R. Greul, G. Hochreiner, G. Syré, C. Tausch, G. Wahl (Linz Hospital, Linz); A.
Chott, H. Hartleb, H. Ludwig, H. Salzer (Wilhelminenspital, Vienna); R. Kocher, G. Leitner, H. Rabl, F. Stangl, R. Stering (Leoben Hospital, Leoben); E. Forsthuber, K. Holzmüller, J. Omann, H. Rogatsch, A. Urbania (Klagenfurt Hospital, Klagenfurt); H. Feichtinger, M. Rottmann, J. Schüller (Rudolfstiftung Hospital, Vienna); O. Braun, F. Burger (Waldviertel Hospital, Horn); G. Bayer-Tiefenbach, K. Mach (Oberwart Hospital, Oberwart); B. Gebhard, W. Rausch (Vöcklabruck Hospital, Vöcklabruck); W. Öhlinger, R. Punzengruber (Amstetten Hospital, Amstetten); W. Döller, E. Melbinger (Wolfsberg Hospital, Wolfsberg); H. Brustmann, P. Riss (Mödling Hospital, Mödling).
References


after 2 years’ adjuvant tamoxifen: combined results of ABCSG trial 8 and ARNO 95 trial.

(p53, p21, pRb and mdm2) and c-erbB-2 with biological markers of proliferation and

anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 10-year

16. Bartlett JM, Brookes CL, Robson T, van de Velde CJ, Billingham LJ, Campbell
FM, et al. Estrogen receptor and progesterone receptor as predictive biomarkers of
response to endocrine therapy: a prospectively powered pathology study in the
Table 1. Baseline characteristics of patients according to Ki67 expression

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ABCSG-8 n = 3,714</th>
<th>Total n = 1,587</th>
<th>Ki67 low n = 1,193</th>
<th>Ki67 high n = 394</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at surgery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.21</td>
</tr>
<tr>
<td>Median, years</td>
<td>63.8</td>
<td>63.6</td>
<td>63.5</td>
<td>64.3</td>
<td></td>
</tr>
<tr>
<td>Range, years</td>
<td>41.4 – 80.5</td>
<td>41.5 – 80.5</td>
<td>41.5 – 80.5</td>
<td>48.3 – 79.8</td>
<td></td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>T1</td>
<td>2,782 (74.9)</td>
<td>1,136 (71.6)</td>
<td>882 (73.9)</td>
<td>254 (64.5)</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>899 (24.2)</td>
<td>434 (27.3)</td>
<td>298 (25.0)</td>
<td>136 (34.5)</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>33 (0.9)</td>
<td>17 (1.1)</td>
<td>13 (1.1)</td>
<td>4 (1.0)</td>
<td></td>
</tr>
<tr>
<td>Nodal status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.14</td>
</tr>
<tr>
<td>Negative</td>
<td>2,770 (74.6)</td>
<td>1,141 (71.9)</td>
<td>875 (73.3)</td>
<td>266 (67.5)</td>
<td></td>
</tr>
<tr>
<td>1 to 3 positive nodes</td>
<td>831 (22.4)</td>
<td>400 (25.2)</td>
<td>285 (23.9)</td>
<td>115 (29.2)</td>
<td></td>
</tr>
<tr>
<td>&gt;4 positive nodes</td>
<td>112 (3.0)</td>
<td>46 (2.9)</td>
<td>33 (2.8)</td>
<td>13 (3.3)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (&lt;0.1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Tumor grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>G1</td>
<td>739 (19.9)</td>
<td>361 (22.7)</td>
<td>328 (27.5)</td>
<td>33 (8.4)</td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>2,811 (75.7)</td>
<td>1,110 (69.9)</td>
<td>776 (65.0)</td>
<td>334 (84.8)</td>
<td></td>
</tr>
<tr>
<td>Gx lobular</td>
<td>164 (4.4)</td>
<td>116 (7.3)</td>
<td>89 (7.5)</td>
<td>27 (6.9)</td>
<td></td>
</tr>
<tr>
<td>Estrogen receptor†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.85</td>
</tr>
<tr>
<td>Negative</td>
<td>46 (1.2)</td>
<td>18 (1.1)</td>
<td>15 (1.3)</td>
<td>3 (0.8)</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>336 (9.0)</td>
<td>131 (8.3)</td>
<td>100 (8.4)</td>
<td>31 (7.9)</td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>1,027 (27.7)</td>
<td>466 (29.4)</td>
<td>351 (29.4)</td>
<td>115 (29.2)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>2,300 (61.9)</td>
<td>972 (61.2)</td>
<td>727 (60.9)</td>
<td>245 (62.2)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>5 (0.1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
Table 1. continued

<table>
<thead>
<tr>
<th>Progesterone receptor†</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>684 (18.4)</td>
<td>299 (18.8)</td>
<td>209 (17.5)</td>
<td>90 (22.8)</td>
</tr>
<tr>
<td>Low</td>
<td>677 (18.2)</td>
<td>298 (18.8)</td>
<td>212 (17.8)</td>
<td>86 (21.8)</td>
</tr>
<tr>
<td>Medium</td>
<td>1,214 (32.7)</td>
<td>522 (32.9)</td>
<td>400 (33.5)</td>
<td>122 (31.0)</td>
</tr>
<tr>
<td>High</td>
<td>1,132 (30.5)</td>
<td>468 (29.5)</td>
<td>372 (31.2)</td>
<td>96 (24.4)</td>
</tr>
<tr>
<td>Unknown</td>
<td>7 (0.2)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HER2‡ (n =1581)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>NA</td>
<td>1,483 (93.8)</td>
<td>1,138 (95.5)</td>
<td>345 (88.7)</td>
</tr>
<tr>
<td>Positive</td>
<td>NA</td>
<td>98 (6.2)</td>
<td>54 (4.5)</td>
<td>44 (11.3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Adjuvant therapy§</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TAM</td>
<td>1,849 (49.8)</td>
<td>785 (49.5)</td>
<td>588 (49.3)</td>
</tr>
<tr>
<td>TAM→ANA</td>
<td>1,865 (50.2)</td>
<td>802 (50.5)</td>
<td>605 (50.7)</td>
</tr>
</tbody>
</table>

*P values were calculated by the chi-square test. Percentages may not total 100 because of rounding.

†Hormone-receptor status was defined by the Reiner score for immunohistochemical staining, which is based on a scale of 10 to 100%, with 10 to 50% indicating low expression of the estrogen and progesterone receptors, 51 to 80% indicating medium expression, and 81 to 100% indicating high expression.

‡Tumors were considered to be HER2 positive if immunohistochemistry was 3+ or if amplified by fluorescent in situ hybridization (more than six \( \text{HER2} \) gene copies per nucleus or a FISH ratio of more than 2.2).

§TAM = tamoxifen for 5 years; TAM→ANA = tamoxifen for 2 years followed by anastrozole for 3 years.
Table 2. Cox proportional hazard models

Univariate analyses

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard ratio for recurrence</th>
<th>95% CI*</th>
<th>P</th>
<th>Hazard ratio for death</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.05</td>
<td>1.03-1.07</td>
<td>&lt;0.0001</td>
<td>1.08</td>
<td>1.05-1.11</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tumor size</td>
<td>2.41</td>
<td>1.83-3.18</td>
<td>&lt;0.0001</td>
<td>3.13</td>
<td>2.23-4.39</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Nodal status</td>
<td>2.03</td>
<td>1.59-2.58</td>
<td>&lt;0.0001</td>
<td>2.30</td>
<td>1.71-3.08</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tumor grade</td>
<td>1.11</td>
<td>0.90-1.37</td>
<td>0.32</td>
<td>1.10</td>
<td>0.84-1.42</td>
<td>0.50</td>
</tr>
<tr>
<td>ER</td>
<td>0.95</td>
<td>0.76-1.18</td>
<td>0.61</td>
<td>1.06</td>
<td>0.79-1.42</td>
<td>0.70</td>
</tr>
<tr>
<td>PgR</td>
<td>0.85</td>
<td>0.74-0.98</td>
<td>0.02</td>
<td>0.93</td>
<td>0.77-1.11</td>
<td>0.41</td>
</tr>
<tr>
<td>HER2</td>
<td>1.55</td>
<td>0.89-2.68</td>
<td>0.12</td>
<td>1.75</td>
<td>0.91-3.37</td>
<td>0.09</td>
</tr>
<tr>
<td>Adjuvant therapy</td>
<td>0.77</td>
<td>0.56-1.06</td>
<td>0.11</td>
<td>1.01</td>
<td>0.68-1.50</td>
<td>0.96</td>
</tr>
<tr>
<td>Ki67</td>
<td>2.28</td>
<td>1.66-3.13</td>
<td>&lt;0.0001</td>
<td>2.15</td>
<td>1.44-3.22</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

Multivariate analyses

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard ratio for recurrence</th>
<th>95% CI</th>
<th>P</th>
<th>Hazard ratio for death</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.05</td>
<td>1.03-1.07</td>
<td>&lt;0.0001</td>
<td>1.07</td>
<td>1.04-1.10</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tumor size</td>
<td>1.86</td>
<td>1.39-2.48</td>
<td>&lt;0.0001</td>
<td>2.30</td>
<td>1.61-3.28</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Nodal status</td>
<td>1.78</td>
<td>1.39-2.29</td>
<td>&lt;0.0001</td>
<td>1.91</td>
<td>1.41-2.59</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tumor grade</td>
<td>0.94</td>
<td>0.75-1.19</td>
<td>0.61</td>
<td>0.95</td>
<td>0.72-1.27</td>
<td>0.75</td>
</tr>
<tr>
<td>ER</td>
<td>0.90</td>
<td>0.71-1.13</td>
<td>0.35</td>
<td>0.97</td>
<td>0.72-1.31</td>
<td>0.84</td>
</tr>
<tr>
<td>PgR</td>
<td>0.89</td>
<td>0.77-1.04</td>
<td>0.14</td>
<td>0.96</td>
<td>0.80-1.16</td>
<td>0.69</td>
</tr>
<tr>
<td>HER2</td>
<td>1.32</td>
<td>0.76-2.31</td>
<td>0.33</td>
<td>1.61</td>
<td>0.83-3.13</td>
<td>0.16</td>
</tr>
<tr>
<td>Adjuvant therapy</td>
<td>0.80</td>
<td>0.58-1.10</td>
<td>0.17</td>
<td>1.03</td>
<td>0.69-1.53</td>
<td>0.89</td>
</tr>
<tr>
<td>Ki67</td>
<td>1.90</td>
<td>1.37-2.64</td>
<td>&lt;0.0001</td>
<td>1.78</td>
<td>1.18-2.70</td>
<td>0.006</td>
</tr>
</tbody>
</table>

*95% CI = 95% confidence interval.

Variables were coded as described in Table 1.
Figure legends

Figure 1. Study flowchart for the process of tumor block and patient selection.

Figure 2. Kaplan-Meier estimates of the probability of recurrence-free survival (A) and overall survival (B) according to dichotomized Ki67 expression (≤10%, >10%) of 1,587 breast cancer patients enrolled in ABCSG-8.

Figure 3. Recurrence-free survival according to adjuvant treatment and Ki67 expression in 1,438 patients with medium or high ER expressing tumors. TAM, tamoxifen alone; TAM→ANA tamoxifen followed by anastrozole.
ABCSG-8
n = 3,714

TAM
n = 1,849

No blocks available
n = 1,009

Submitted blocks
n = 840

Excluded
n = 55
No invasive tumor
n = 34
Section detached
n = 6
Low material
n = 15

Centrally assessed for Ki67
n = 785

Final sample
n = 1,587

TAM → ANA
n = 1,865

No blocks available
n = 1,009

Submitted blocks
n = 856

Excluded
n = 54
No invasive tumor
n = 30
Section detached
n = 3
Low material
n = 21

Centrally assessed for Ki67
n = 802

Final sample
n = 1,587
A

Recurrence-free survival %

Log rank p<0.0001

Ki67 low (92 events)

Ki67 high (65 events)

No. at risk

Years

1193 1186 1162 1120 1059 779

394 388 373 353 318 233

B

Overall survival %

Log rank p<0.0001

Ki67 low (58 events)

Ki67 high (40 events)

No. at risk

Years

1193 1186 1167 1128 1076 810

394 390 379 368 337 256
Interaction p=0.03

- Ki67 low / TAM (52 events)
- Ki67 low / TAM→ANA (31 events)
- Ki67 high / TAM (29 events)
- Ki67 high / TAM→ANA (29 events)

<table>
<thead>
<tr>
<th>No. at risk</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ki67 low / TAM</td>
<td>531</td>
<td>527</td>
<td>518</td>
<td>493</td>
<td>465</td>
<td>319</td>
</tr>
<tr>
<td>Ki67 low / TAM→ANA</td>
<td>547</td>
<td>544</td>
<td>532</td>
<td>516</td>
<td>489</td>
<td>378</td>
</tr>
<tr>
<td>Ki67 high / TAM</td>
<td>182</td>
<td>180</td>
<td>175</td>
<td>165</td>
<td>147</td>
<td>99</td>
</tr>
<tr>
<td>Ki67 high / TAM→ANA</td>
<td>178</td>
<td>175</td>
<td>167</td>
<td>161</td>
<td>143</td>
<td>111</td>
</tr>
</tbody>
</table>

Recurrence-free survival %

Years
Adjuvant sequencing of tamoxifen and anastrozole is superior to tamoxifen alone in postmenopausal women with low proliferating breast cancer

Zsuzsanna Bago-Horvath, Margaretha Rudas, Peter Dubsky, et al.

*Clin Cancer Res* Published OnlineFirst October 13, 2011.