Estrogen receptor, Progesterone receptor, HER2 status and Ki67 index and responsiveness to adjuvant tamoxifen in postmenopausal high-risk breast cancer patients enrolled in the DBCG 77C trial

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Received 15 October 2013; received in revised form 20 February 2014; accepted 23 February 2014
Available online 24 March 2014

Abstract Background: The DBCG 77C trial compared one year of tamoxifen in postmenopausal, steroid-receptor unknown, high-risk breast cancer patients to no adjuvant systemic therapy. After a potential follow-up of 30 years we report overall efficacy of the study and results according to subtypes subsequently assessed by immunohistochemistry and fluorescent in situ hybridisation (FISH).

Methods: Between 1977 and 1982, 1716 postmenopausal patients with tumours larger than 5 cm or positive axillary nodes were randomly assigned to no systemic therapy or tamoxifen 30 mg daily for one year. Archival tumour tissue from 1515 patients was analysed and the hormone receptor positive (estrogen receptor (ER) and/or progesterone receptor (PR)) cancers were defined as luminal A if Ki67 low and HER2-negative; as luminal B if Ki67 high or HER2-positive; and otherwise as non-luminal-HER2 positive or triple negative.

Findings: In the intent-to-treat (ITT) population one year of tamoxifen improved the disease-free-survival (DFS) (hazard ratio (HR) = 0.87; 95% confidence interval (CI) 0.77–0.98), the
Breast Cancer Recurrence Rate (BCRR) (HR = 0.79; 0.69–0.90) and reduced the breast-cancer-specific-mortality (BCM) (HR = 0.83; 0.73–0.93). BCRR were improved significantly by tamoxifen in luminal A (HR = 0.66; 0.53–0.84) and luminal B/HER2– (HR = 0.54; 0.39–0.74) but not in the other subsets, and with similar results for BCM with 30 years follow-up.

**Interpretation:** One year of treatment with tamoxifen significantly improves BCRR and BCM in postmenopausal patients with ER positive breast cancers. The relative benefit from tamoxifen was not significantly different in luminal A and B subtypes.

**Funding:** The Danish Breast Cancer Cooperative Group (DBCG) prepared the original protocol (DBCG 77C) and was the sponsor of the study. Funding was not provided to the participating departments. The biomarker study was supported by grants from the Clinical Institute, Odense University.

1. **Introduction**

The recently updated meta-analysis regarding hormone receptors and long term effect of adjuvant tamoxifen, the Early Breast Cancer Trialists’ Collaborative Group (EBCTCG) [1], found estrogen receptor (ER) status to be the only important predictor of reduced 15-year risks of breast cancer recurrence and death. Luminal cancers are biologically distinct from ER negative cancers as demonstrated by gene expression profiles more than a decade ago [2]. Specific biological characteristics or treatable targets have not enabled a further breakdown of the large group of luminal cancers and consequently the luminal group was divided according to proliferation genes [3–6]. Gene expression profiling has only been carried out in a few of the adjuvant tamoxifen trials. Using the PAM50 classifier Chia et al., demonstrated a statistically significant benefit of adjuvant tamoxifen in the NCIC MA.12 trial among patients with luminal subtypes (hazard ratio (HR) = 0.52; confidence interval (CI) (95%), 0.32–0.86; \( p = .009 \)) [6]. The classifier was superior to conventional local and central immunohistochemical (IHC) ER assessment for both prognosis and more importantly for prediction of benefit to adjuvant tamoxifen compared with placebo. Jerevall et al. [7] who used a molecular grade index (a gene expression assay comprised of five genes related to proliferation) on tumour blocks from the placebo controlled randomized Stockholm trial supported the predictive value of the proliferation index on breast cancer outcome, both in treated and untreated patients.

An IHC determined surrogate marker for proliferation, the Ki67-labeling index, has been investigated for its ability to predict prognosis and response to endocrine therapy. Cheang et al. [8] subtyped breast cancer specimens both by gene expression profiles (the PAM50 classifier) and by IHC including ER, progesterone receptor (PR), HER2 and Ki67-labeling index. They showed that the expression of ER, PR, HER2 and the Ki67 index appeared to distinguish luminal A from luminal B breast cancer subtypes and that the patients with luminal B tumours had an increased risk of breast cancer relapse and death in comparison with the more common luminal A subtype.

The predictive value of the Ki67 labeling index has been evaluated in three large adjuvant trials (BIG-98 [9], ABCSG [10] and ATAC [11]) comparing tamoxifen to an aromatase inhibitor or a sequence of the two drugs. The results were however inconclusive and a statistical significant heterogeneity between treatment and Ki67 status was not detected in any of the three trials.

At the 2011 St Gallen International Breast Cancer Conference the panel agreed that therapeutic decisions could be made based on the approximated recognition of the intrinsic subtypes of breast cancer defined by the expression of ER, PR, Ki67 and HER2 [12]. The panel concluded, that the cut-point for Ki67 should be 14% based on previous comparison with the PAM50 methodology [13,8]. At the 2013 St Gallen International Breast Cancer Conference the panel could not reach an agreement regarding a cut-point [14].

The DBCG 77C randomized high-risk, receptor unknown, postmenopausal breast-cancer patients to one year of tamoxifen or to observation. For the first time we report the overall results from the study and the predictive value of intrinsic subtypes compared with ER alone.

2. **Materials and methods**

The DBCG 77C tamoxifen trial was an open-labelled randomized trial [15]. The organization of DBCG and the design of the clinical database have previously been described in detail [16].

2.1. **Patients and procedures**

Between November 1977 and December 1982 1,716 eligible patients from centres in Denmark were randomized either to tamoxifen 10 mg three times per day (\( n = 868 \)) or to observation (\( n = 848 \)). Criteria of
eligibility included postmenopausal status (amenorrhoea for at least 5 years), either node positive disease, or a primary tumour larger than 5 cm in diameter, or invasion of the tumour into skin or deep fascia, no evidence of advanced disease by physical examination, radiography of the chest and bone or bone scintigraphy and no previous or concomitant malignant disease.

Classification of histologic type (ductal carcinoma not otherwise specified, lobular carcinomas or other type), histologic grade according to Bloom and Richardson, measurement of size, invasion of the tumour into skin or deep fascia and numbers of removed and positive axillary lymph nodes were evaluated and reported prospectively. The 27 departments of pathology participating in the DBCG trials in the time period 1977–82 performed the histopathological examinations.

Total mastectomy and lower axillary dissection followed by radiotherapy to the chest wall and regional lymph nodes were mandatory, and two different radiotherapy schedules were used (40.92 Gy in 22 fractions, 5 fractions per week, or 36.60 Gy in 12 fractions, 2 fractions per week). Treatment related adverse events and findings on clinical examination were recorded every third month during the first and second year, every six months during the third to fifth year and thereafter annually to a total of 10 years. In addition data on long-term survival and causes of death were collected through linkage to the Danish Central Population Registry and the Danish Register of Causes of Death [17].

There were considered HER2(3+) positive if more than 50% of tumour cells had a strong positive membranous reaction. HER2 fluorescent in situ hybridisation (FISH) (Dako, Glostrup, Denmark) was performed according to the manufacturers’ manual if 10–50% tumour cells stained IHC positive – and thereby equal to HER2(2+). These tumours were considered FISH positive if HER2: CEN17 ratio was ≥2.

For Ki67 IHC the MIB1 antibody (Dako M7240) was applied (1:200). Deparaffination and antigen retrieval were performed with Dako Target Retrieval Solutions High pH for 40 min (standard 20) at 97° (Code K6002) using the Dako pretreatment (PT) link. After blocking of endogenous peroxidase activity with Dako EnVision Flex+(Dako K8002) for 5 min, tissue sections were incubated with primary antibody diluted in Dako antibodydiluent (Dako K8006) for 40 min at room temperature. The reaction was visualized using Dako EnvisionFlex + mouselink for 15 min followed by diamobenzidine (DAB) for 10 min. The sections were counterstained with haematoxylin for 1 min. Evaluation of the Ki67 IHC staining was performed by bright field microscopy. A semi-quantitative evaluation of Ki67 positive tumour cells was given in percentage focusing on the invasive tumour front and Ki67 IHC hot spots. Only nuclear staining of the invasive tumour cells was analysed. Ki67 <14% was considered low-positive and ≥14% high positive.

The patients were subtyped as follows; Luminal A: Patients with ER+ or PR+, HER2-negative and Ki67 <14% tumours. Luminal B/HER2-negative: Patients with ER+ or PR+, HER2-negative and Ki67 ≥14% tumours. Luminal B/HER2-positive: Patients with ER+ or PR+, HER2-positive and Ki67 ≥14% tumours. Non-luminal/HER2-positive: Patients with HER2-positive, ER−, PR− negative tumours and patients with ER−, PR− and HER2-negative tumours were defined triple negative (TN) [8,19].

2.3. Statistical analysis

The DBCG Data Center undertook central review, querying and analysis of data. Follow-up time was quantified in terms of a Kaplan–Meier estimate of potential follow-up [20]. Disease-free-survival (DFS) was defined as the duration of survival without locoregional recurrence, distant metastases, contralateral invasive breast cancer, second primary non-breast invasive cancer or death irrespective of the cause. Cumulative incidences in the presence of competing risk were estimated for the risk of death from breast cancer (BCM) and for the breast cancer recurrence rate (BCRR) comprising invasive locoregional recurrence, distant metastases or death from breast cancer as first event [21]. The main study is reported as an ITT analysis with the predefined DFS and BCM as end-point in an unadjusted analysis. Secondary comparisons, including comparisons between ER positive and negative patients, and IHC-defined sub-sets were not specified in advance but analysed in the assessable (regarding markers) per-protocol population for BCRR and BCM in multivariate analyses. For multivariate analysis tumour size, nodal status (positive nodes combined with proportion of positive nodes examined), histological type and grade, invasion of deep fascia or skin, ER, PR, Ki67, HER2 and treatment were included. Age was not statistically significant (p = .92 for BCM and p = .39 for recurrence) and was not considered further. The log-rank test, standard Cox regression, competing-risks regression models according to the method of Fine and Gray [22] and the Wald test were applied. The assumptions of
proportional hazards were assessed by Schoenfeld residuals and by including time-dependent variables in the models. Interactions between treatment and the covariates ER, PR, Ki67, HER2 and subtypes were investigated in separate models. The Kaplan–Meier method was used to estimate the proportion of patients who continued endocrine therapy [23]. Patients adherent to therapy were censored at the date of recurrence, death or other reason for off-study. Associations between assessable as well as subtypes and other characteristics (excluding unknowns) were analysed by chi-square test. P-values are two-tailed. Statistical analyses were done with the SAS 9.2 statistical software (SAS Institute, Inc., Cary, NC) and the STATA v11.0 (StataCorp, Texas, United States of America [USA]).

3. Results

Fig. 1 shows the assignment to the two treatment arms. A complete follow-up for survival until 1st June 2011 was achieved for all patients. Patients included in the per-protocol had at least one registration of treatment/follow-up after randomization and 75 patients were excluded in the per-protocol analysis. Treatment adherence in the ITT population was 81.2% (78.4–83.7) at 36 weeks and 74.4% (71.3–77.2) completed the pre-planned treatment.

End-point events in the DBCG 77C trial according to treatment for both the ITT and the assessable per-protocol population can be seen in Table 1. The randomization was well balanced with no significant differences of the classic variables between the control and the tamoxifen treated groups (data not shown).

Formalin-fixed, paraffin-embedded primary breast tumour tissue blocks were available from 1548 (90%) of the 1716 participants enrolled and 1428 were assessable for ER, PR, HER2 and Ki67. The assessable per-protocol patients (N = 1364) were not significantly different from the remaining 352 with regard to age, tumour size, malignancy grade, proportion of positive nodes, DFS (p = .19) or BCM, (p = .70).

3.1. DFS, BCRR and BCM in the intention-to-treat (ITT) population

This analysis was conducted more than 28 years after closure of recruitment and had a median estimated potential follow-up of 10 years for recurrence and
30 years for survival. As first event, 576 and 553 DFS events were observed in the (ITT) control and tamoxifen groups, respectively (Table 1). DFS (Fig. 2A) and BCRR (not shown) were significantly improved for the tamoxifen group compared to the control group (HR = 0.87; 95% CI, 0.77–0.98; \(p = .02\)) and (HR = 0.79; 95% CI, 0.69–0.90; \(p = .0005\)), respectively. In total, 1674 of 1716 patients had died, and patients who were treated with tamoxifen for one year had a significantly reduced BCM with a HR of 0.83 (95% CI, 0.73–0.93; \(p = .002\)) (Fig. 2B). The HR for the BCRR and BCM (\(N = 1364\)) adjusted for baseline characteristics was 0.74 (95% CI, 0.64–0.86; \(p < .0001\)) and 0.81 (95% CI, 0.70–0.93; \(p = .001\)), respectively (Fig. 5).

### Table 1

<table>
<thead>
<tr>
<th>Event</th>
<th>ITT Control ((N = 848))</th>
<th>ITT Tamoxifen ((N = 868))</th>
<th>Per-Protocol*</th>
<th>Per-Protocol TC ((N = 678))</th>
<th>Per-Protocol TC TAM ((N = 686))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>Disease-free survival (DFS) events</td>
<td>576 67.9</td>
<td>553 63.7</td>
<td>469 63.2</td>
<td>441 64.3</td>
<td>441 64.3</td>
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<tr>
<td>Local/regional invasive recurrence only</td>
<td>62 7.3</td>
<td>54 6.2</td>
<td>53 7.8</td>
<td>42 6.1</td>
<td>61</td>
</tr>
<tr>
<td>Distant recurrence</td>
<td>327 38.6</td>
<td>276 31.8</td>
<td>271 40.0</td>
<td>222 32.4</td>
<td>32.4</td>
</tr>
<tr>
<td>Invasive contralateral breast cancer</td>
<td>16 1.9</td>
<td>21 2.4</td>
<td>11 1.6</td>
<td>15 2.2</td>
<td>2.2</td>
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<tr>
<td>Second primary cancer (invasive non-breast)</td>
<td>18 2.1</td>
<td>27 3.1</td>
<td>14 2.1</td>
<td>23 3.4</td>
<td>3.4</td>
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<tr>
<td>Death, first event</td>
<td>153 18.0</td>
<td>175 20.2</td>
<td>120 17.7</td>
<td>139 20.3</td>
<td>20.3</td>
</tr>
<tr>
<td>DFS at 10 years</td>
<td>27.4 30.9</td>
<td>30.9</td>
<td>27.1 31.9</td>
<td>31.9</td>
<td></td>
</tr>
<tr>
<td>Cumulative incidence of recurrence at 10 years</td>
<td>59.7 51.2</td>
<td>51.2</td>
<td>60.3 50.7</td>
<td>50.7</td>
<td></td>
</tr>
<tr>
<td>Death, any cause</td>
<td>826 97.4</td>
<td>848 97.7</td>
<td>660 97.3</td>
<td>669 97.5</td>
<td>97.5</td>
</tr>
<tr>
<td>Death, breast cancer after 30 years</td>
<td>536 63.1</td>
<td>480 55.3</td>
<td>431 63.4</td>
<td>376 54.8</td>
<td>54.8</td>
</tr>
<tr>
<td>Overall survival at 10 years</td>
<td>32.8 36.4</td>
<td>36.4</td>
<td>32.0 36.3</td>
<td>36.3</td>
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</table>

*Per-protocol patients with ER, PR, Ki67 and HER2 test results.

The treatment effect was not statistically significantly different over time.

### 3.2. BCRR and BCM in the per-protocol (PP) population according to subtypes

Of the 1364 patients with available immunohistochemical information, 444 (33%) were classified as ER negative (Table 2), hereof 392 double negative (ER/PR-negative) and 52 as ER− and PR+.

The efficacy of treatment was restricted to ER+ patients (Fig. 3A and B) and patients with ER/PR +/+ (\(N = 553\)) and ER/PR +/− (\(N = 376\)) had similar benefit regarding recurrences from treatment with...
tamoxifen (HR = 0.61; 95% CI, 0.48–0.78; \( p < .0001 \)) and (HR = 0.62; 95% CI, 0.47–0.83; \( p = .001 \)), respectively (Fig. 5A).

**Table 2**

Patient and tumour characteristics by molecular subset in the per-protocol population with known IHC variables.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PP (N = 1364)</th>
<th>Luminal A</th>
<th>Lum B/HER2–</th>
<th>Lum B/HER2+</th>
<th>HER2+</th>
<th>TN</th>
<th>p-value</th>
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<tr>
<td>Age</td>
<td>661</td>
<td>48%</td>
<td>240</td>
<td>18</td>
<td>71</td>
<td>5</td>
<td>129</td>
</tr>
<tr>
<td>40–59 Years</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>60–64 Years</td>
<td></td>
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<tr>
<td>65–69 Years</td>
<td></td>
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<tr>
<td>70–74 Years</td>
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<tr>
<td>75–Years</td>
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<td>Lymph node status</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative (174)</td>
<td>97</td>
<td>14.7%</td>
<td>26</td>
<td>10.8%</td>
<td>7</td>
<td>9.9</td>
<td>12</td>
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<tr>
<td>1–3 positive (748)</td>
<td>384</td>
<td>58.0%</td>
<td>122</td>
<td>50.8%</td>
<td>37</td>
<td>52.1</td>
<td>69</td>
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<tr>
<td>&gt;4 positive (390)</td>
<td>152</td>
<td>23.0%</td>
<td>87</td>
<td>36.3%</td>
<td>22</td>
<td>31.0</td>
<td>45</td>
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<tr>
<td>Unknown (52)</td>
<td>28</td>
<td>4.2%</td>
<td>5</td>
<td>2.1%</td>
<td>2</td>
<td>7.0</td>
<td>3</td>
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<tr>
<td>Tumour size</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>0 – 2 cm (353)</td>
<td>190</td>
<td>28.7%</td>
<td>60</td>
<td>25.0%</td>
<td>16</td>
<td>22.5</td>
<td>27</td>
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<tr>
<td>2 cm – 5 cm (753)</td>
<td>346</td>
<td>55.1%</td>
<td>139</td>
<td>57.9%</td>
<td>43</td>
<td>60.6</td>
<td>68</td>
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<tr>
<td>&gt;5 cm (253)</td>
<td>103</td>
<td>15.6%</td>
<td>41</td>
<td>17.1%</td>
<td>12</td>
<td>16.9</td>
<td>34</td>
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<tr>
<td>Unknown (5)</td>
<td>4</td>
<td>0.6%</td>
<td>0</td>
<td>0%</td>
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<td>0</td>
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<td>Histologic type</td>
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<tr>
<td>Ductal carcinoma (1189)</td>
<td>567</td>
<td>85.8%</td>
<td>222</td>
<td>92.5%</td>
<td>66</td>
<td>93.0</td>
<td>114</td>
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<td>Lobular carcinoma (74)</td>
<td>48</td>
<td>7.3%</td>
<td>7</td>
<td>2.9%</td>
<td>3</td>
<td>4.2</td>
<td>5</td>
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<tr>
<td>Other carcinomas (101)</td>
<td>46</td>
<td>6.9%</td>
<td>11</td>
<td>4.6%</td>
<td>2</td>
<td>2.8</td>
<td>10</td>
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<tr>
<td>Malignancy grade</td>
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<tr>
<td>Grade I (352)</td>
<td>253</td>
<td>44.6%</td>
<td>43</td>
<td>19.4%</td>
<td>9</td>
<td>13.6</td>
<td>15</td>
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<tr>
<td>Grade II (629)</td>
<td>278</td>
<td>49.0%</td>
<td>130</td>
<td>58.6%</td>
<td>43</td>
<td>65.2</td>
<td>64</td>
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<tr>
<td>Grade III (202)</td>
<td>35</td>
<td>6.2%</td>
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<td>22.1%</td>
<td>13</td>
<td>19.7</td>
<td>34</td>
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<td>Unknown (6)</td>
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<td>0</td>
<td>0%</td>
<td>1</td>
<td>1.5</td>
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<tr>
<td>Ki67</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Low &lt;14% (856)</td>
<td>661</td>
<td>100%</td>
<td>27</td>
<td>38.0%</td>
<td>43</td>
<td>33.3</td>
<td>125</td>
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<tr>
<td>High ≥14% (508)</td>
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<td>0%</td>
<td>0</td>
<td>100%</td>
<td>44</td>
<td>62.0</td>
<td>86</td>
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<tr>
<td>HER2</td>
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<tr>
<td>&gt;50%, &gt;10% and ≤50% and amplified (200)</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>100%</td>
<td>71</td>
<td>100</td>
<td>129</td>
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<tr>
<td>Negative (1164)</td>
<td>661</td>
<td>100%</td>
<td>240</td>
<td>100%</td>
<td>129</td>
<td>100</td>
<td>263</td>
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<td>ER receptor status</td>
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<tr>
<td>Positive (920)</td>
<td>624</td>
<td>94.4%</td>
<td>226</td>
<td>94.2%</td>
<td>70</td>
<td>98.6</td>
<td>0</td>
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<tr>
<td>Negative (444)</td>
<td>37</td>
<td>5.6%</td>
<td>14</td>
<td>5.8%</td>
<td>1</td>
<td>1.4</td>
<td>129</td>
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<td>PgR</td>
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<tr>
<td>Positive (759)</td>
<td>434</td>
<td>65.7%</td>
<td>152</td>
<td>63.3%</td>
<td>19</td>
<td>26.8</td>
<td>0</td>
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<tr>
<td>Negative (605)</td>
<td>227</td>
<td>34.3%</td>
<td>88</td>
<td>36.7%</td>
<td>52</td>
<td>73.2</td>
<td>129</td>
</tr>
</tbody>
</table>

* Ductal carcinomas only.
† Between all five groups.
‡ Between LumA and LumB/HER2–.

HER2 and Ki67 were used to subtype the 972 hormone receptor positive tumours. We classified 661 (48%) as luminal A and 240 (18%) as luminal B/HER2– and 71 (5%) as luminal B/HER2+ (Table 2). Among the remaining 392 tumours, we classified 129 (9%) as non-luminal/HER2-positive and 263 (19%) as triple negative. Patients with luminal A had lower number of positive nodes (\( p = .0006 \)), lower grade (\( p < .0001 \)) and were more often classified as lobular type (\( p = .02 \)) compared to luminal B/HER2– (Table 2). Patients in the control group with luminal A and B/HER2–, had significant different breast cancer recurrence rates (55.1% and 69.1% at 10 years, respectively) which were significantly decreased in the tamoxifen treated luminal groups (40.8% and 57.0%, respectively) (Fig. 4A and B). After 30 years, 57.9% of the luminal A control group had died from breast cancer while 69.0% of the Luminal B/HER2– patients in the control group had died versus 57.5% in the tamoxifen treated group (Fig. 4C and D). Also the small luminal B/HER2+ group of patients had a treatment benefit from tamoxifen although not statistically significant (Fig. 5A and B).
There was no significant difference between luminal A versus luminal B/HER2− versus luminal B/HER2+ patients \( (p = .51 \text{ for BCRR and } P = .87 \text{ for BCM}) \) (Fig. 5 A and B).

The poor outcome seen in patients with non-luminal-HER2-positiv and TN tumours was not modified by treatment (Figs. 4 and 5).

4. Discussion

The DBCG 77C tamoxifen trial shows a life-lasting benefit from one year of tamoxifen as compared to no adjuvant systemic therapy and notably survival benefits achieved at 10 years persisted. With 30 years potential follow-up 1674 of the 1716 patients had died. In the 30-years breast-cancer-specific analysis the number of events has allowed us to detect a 17\% decrease in BCM\[24\] despite the fact that 1/3 of the patients had ER-negative tumours \( (HR = 0.83; \text{ 95\% CI, 0.73–0.93}) \). In agreement with the EBCTCG meta-analysis\[1\] ER status was the only factor that predicted the reductions by tamoxifen observed in BCRR and BCM. When added to the ER status, the PR measurement gave no additional information regarding endocrine-responsive-ness as previously reported by the EBCTCG\[1\]. When ER-positive patients were divided into subtypes by PR, HER2 and Ki67 the two luminal subtypes (A and B) showed significantly different recurrence rates and breast-cancer mortality in accordance with Cheang et al.\[8\] and others\[6,9,10\]. We found no statistical evidence of heterogeneity in the effect of tamoxifen between the two luminal subtypes, and it will be interesting to see whether five years of treatment with tamoxifen or an aromatase inhibitor will have a more pronounced effect in one of the luminal subtypes.

It has been argued that the segregation of ER-positive tumours is a "pseudo" explanation for what is really a continuum of ER-positive cancer forms\[3,25\]. Furthermore, it has been estimated that a substantial part of current hormone receptor testing worldwide may be inaccurate. It has recently been shown\[6\] that intrinsic subtype classification with the PAM50 assay within the context of the randomized MA.12 study was superior to conventional local and central IHC ER assessment. This was true in prognosis and more important in the prediction of benefit from adjuvant tamoxifen compared with placebo.

The proliferation marker Ki67 also forms a continuum and is used to distinguish between Luminal A and B. But the segregation is based on a arbitrarily set and non-validated cut-off values\[26,27\]. In this study, we used Ki67 as proliferation marker with a cut point of 14\% in agreement with the 2011 St Gallen guidelines\[12\] but we agree that the cut point has to be validated and that guidelines – like the one recently published from Dowsett et al.\[28\] – are needed before implementation in daily clinical practice. At present, an acceptable reproducibility of Ki67 particular near the cut point seems almost unachievable\[29\].

Gene expression profiles, i.e. PAM50, are more reproducible but less accessible leading to a considerable interest in detailed subtype-modelling by IHC. Recently, Prat et al.\[30\] concluded that semiquantitative IHC expression of PR adds prognostic value to the current IHC-based luminal A definition by improving the identification of breast cancers with good outcome. The new
proposed IHC-based definition of luminal A tumours was PR more than 20% in the ER positive/HER2 negative/Ki-67 less than 14% group. Our data were unable to confirm that conclusion, as we for PR used 10% as the cut point for distinguishing between positive and negative specimens.

Patients with HER2-positive tumours – luminal as non-luminal – did not benefit significantly from treatment with tamoxifen in accordance with present days consensus that this group should be offered trastuzumab-based adjuvant treatment in addition to standard endocrine therapy. But the luminal B/HER2-positive group is small, and there is no significant difference between the three luminal groups (BCM; \( p = 0.87 \)).

In this study there is a significant interaction between treatment effect and ER status as expected. Patients with less than 10% of ER positive cells experienced a less favourable outcome with the use of tamoxifen as could be expected. Thus, our results are not in accordance with the just recently introduced and recommended cut point [31]. As the proportion of patients with ER-values between 1% and 10% in this material is unknown, but in the Danish breast cancer population in general is <1%, the results are unlikely to changes even if we had used 1% as cut-point.
There are some considerations regarding aroma-
terming, and recently also a survival benefit was found
positive postmenopausal patients is an aromatase inhib-
proportional estimates, which by the EBCTCG [1]
treatment with just one year of tamoxifen induces a long
erally poor prognosis and were treated with tamoxifen
lasting (more than 25 years) survival benefit in patients
The optimal dose of tamoxifen used
to treat breast cancer is unclear and seems to depend on
have shown to be very robust across different prognostic
Overall, no randomized trials have shown higher benefit
In conclusion, ER-status remains the only IHC deter-
in the United States, 20 mg daily has been used exclusively.
Overall, no randomized trials have shown higher benefit
with higher doses of tamoxifen, but a relationship can of
course not be excluded — especially not with different
Today the standard endocrine-treatment for ER-
positive postmenopausal patients is an aromatase inhibitor
for 5 years. In several studies this treatment as com-
pared to tamoxifen has given a significantly reduced recurrences and recently also a survival benefit was found
resection of breast cancer and with pronounced side-effects from endocrine
treatment.
In the DBCG 77C tamoxifen trial, patients had a gen-
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In the DBCG 77C tamoxifen trial, patients had a gen-
C24
Conflict of interest statement
None declared.

Contributors
All authors reviewed the data analyses, contributed
to the writing of the report, made final decisions on all
parts of the report, and approved the final version of
the submitted report. BE participated in study design.
BE, JA and DN enrolled patients. MJ undertook
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Fig. 5. Forest plot: Treatment effect (A) Breast Cancer Recurrence Rate (BCRR) and (B) Breast Cancer Mortality (BCM) according to Oestrogen,
Progesterone and HER2 Receptor Status, Ki67 Index and Breast Cancer Subtypes. Lum A: Luminal A, Lum B HER2+: Luminal B HER2+, TN: Triple negative,
Non-l/HER2+: ER/PR negative and HER2+, HER2A: Altered, HER2N: Normal.

In the DBCG 77C tamoxifen trial, patients had a gen-
earlly poor prognosis and were treated with tamoxifen
30 mg for one year instead of today’s standard of
20 mg for 5–10 years. In this study, we report the results
in proportional estimates, which by the EBCTCG [1]
have shown to be very robust across different prognostic
groups of patients. The optimal dose of tamoxifen used
to treat breast cancer is unclear and seems to depend on
the country of origin and of the original trials. In the
United Kingdom, 20 or 40 mg daily was recommended,
in Canada and Denmark, 30 mg daily was used, but in
the United States, 20 mg daily has been used exclusively.
Overall, no randomized trials have shown higher benefit
with higher doses of tamoxifen, but a relationship can of
course not be excluded — especially not with different
treatment durations.

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Acknowledgement

We thank Tine Rudbeck for excellent technical assistance with the HER2 FISH.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejca.2014.02.022.

References
