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Clinical Cancer Research

High-Risk Premenopausal Luminal A Breast Cancer Patients Derive no Benefit from Adjuvant Cyclophosphamide-based Chemotherapy: Results from the DBCG77B Clinical Trial

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Abstract

Purpose: Luminal A breast cancers have better prognosis than other molecular subtypes. Luminal A cancers may also be insensitive to adjuvant chemotherapy, although there is little high-level evidence to confirm this concept. The primary hypothesis in this formal prospective–retrospective analysis was to assess interaction between subtype (Luminal A vs. other) and treatment (chemotherapy vs. not) for the primary endpoint (10-year invasive disease-free survival) of a breast cancer trial randomizing women to adjuvant chemotherapy, analyzed in multivariate Cox proportional hazards models using the Wald interaction test.

Experimental Design: The Danish Breast Cancer Cooperative Group 77B clinical trial randomized 1,072 premenopausal women to no systematic treatment (control), levamisole, cyclophosphamide, or cyclophosphamide–methotrexate–fluorouracil arms. All arms included radiotherapy but no endocrine therapy. Researchers with no access to clinical data performed intrinsic subtype analysis on tissue microarrays using published immunohistochemical methods based on estrogen receptor, progesterone receptor, HER2, Ki67, and basal markers.

Results: Patients ($n = 709$) had tissue available; chemotherapy benefit in these patients was similar to the original trial (HR, 0.56). Immunohistochemistry classified 165 as Luminal A, 319 Luminal B, 58 HER2-enriched, and 82 core basal (among 91 triple-negative). Patients with Luminal A breast tumors did not benefit from chemotherapy [HR, 1.06; 95% confidence interval (CI), 0.53–2.14; $P = 0.86$], whereas patients with non–luminal A subtypes did (HR, 0.50; 95% CI, 0.38-0.66; $P < 0.001$; $P_{interaction} = 0.048$).

Conclusions: In a prospective–retrospective analysis of a randomized trial, patients with Luminal A breast cancers did not benefit from adjuvant cyclophosphamide-based chemotherapy. *Clin Cancer Res; 1–8. ©2016 AACR.*

Introduction

The Luminal A, Luminal B, HER2-enriched (HER2E), and basal-like intrinsic molecular subtypes of breast cancer were initially discovered on microarrays (1) but can be detected with reasonable accuracy using immunohistochemistry panels (2, 3). Of particular clinical importance is the Luminal A subtype, characterized by high expression of estrogen and progesterone receptors (ER, PR) but low expression of proliferation markers (4).

Multiple studies have demonstrated the good prognosis of Luminal A tumors, including formal prospective-retrospective studies of clinical trials where women received endocrine therapy but not chemotherapy (5, 6). Conventional chemotherapies target replicating cells, and Luminal A tumors express low levels of proliferation genes, providing a theoretical basis for the concept that Luminal A status might predict lack of chemotherapy benefit. While cohort and neoadjuvant studies support this concept (7, 8), the highest level of evidence requires formal interaction testing on clinical trials randomizing women to chemotherapy versus no chemotherapy, against survival endpoints. However, material from such trials is difficult to obtain. The benefit of adjuvant chemotherapy was proven by randomized trials reported in the 1980s (9); since then, most breast cancer studies have randomized among different chemotherapy regimens without including no-chemotherapy arms. The relevant older studies generally did not retain tissue blocks; even among the few that did, collection was incomplete and/or materials were consumed for other studies (10), rendering modern biomarker subset analyses underpowered.

Some materials were available from Danish Breast Cancer Cooperative Group (DBCG) trial 77B, which demonstrated that classical cyclophosphamide–methotrexate–fluorouracil (CMF) and oral single-agent cyclophosphamide (C) significantly reduce recurrence and mortality in premenopausal patients with high-risk early breast cancer treated after effective local therapy (11). Similar results were observed in the first adjuvant Milan trial and NSABP B-20, all consistent with the Early Breast Cancer Trialists' Collaborative Group meta-analysis (12–14). The survival gain was fostered 5 to 10 years after randomization and persisted

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Translational Relevance

Luminal A is the most common subtype of human breast cancer, with the best prognosis. Clinically high-risk cases are routinely treated with adjuvant chemotherapy, which may not benefit such tumors with their low proliferation rate. High-level evidence to test the value of chemotherapy requires an interaction test on a randomized clinical trial relating subtype to chemotherapy. Trials with appropriate randomization were all completed long before molecular subtyping became available. Using a formal prospective–retrospective design applying published assays to tissue from a completed trial, we find that Luminal A patients do not benefit from cyclophosphamide-based chemotherapy, whereas all other subtypes do. This provides level 2 evidence that widely available immunohistochemical methods predict need for chemotherapy and suggests that the population of women with Luminal A breast cancer who may not require chemotherapy could extend to a clinically higher risk group (node-positive or large tumors, even without endocrine therapy).

beyond 25 years, but regardless of treatment one fourth of these patients died within 5 years of randomization. Among those who did not receive adjuvant chemotherapy just over a quarter were alive 25 years later. The relative benefits obtained from chemotherapy were achieved irrespective of age and number of positive lymph nodes. DBCG77B has the potential to extend knowledge about chemotherapy value in Luminal A tumors because, unlike in other studies attempting to identify a low-risk molecular group, patients were premenopausal, clinically high-risk (mostly node-positive) and received no endocrine therapy. Entry criteria were agnostic to ER and HER2 status, so all intrinsic subtypes were included. Fortunately, from this trial, tissue microarrays (TMA) had been constructed from a set of Danish patients' surgical excision blocks. Using these materials, we tested the hypothesis that patients with immunohistochemically defined Luminal A tumors derive no benefit from chemotherapy.

Materials and Methods

Study design

This was a formal prospective–retrospective study following principles described by Simon and colleagues (15) and ReMARK guidelines (16). One set of researchers applied a fully prespecified classifier onto materials from a phase III randomized clinical trial. Results were then related to patient outcome by the clinical trial group's statistical office independently executing a prespecified statistical plan.

DBCG77B study arms and endpoints

The DBCG77B trial included premenopausal women who underwent complete resection for unilateral, invasive adenocarcinoma of the breast by mastectomy with axillary sampling or clearance (level I and part of level II). Patients were required to have axillary lymph node metastases, tumors > 5 cm, or invasion of the deep fascia without distant metastasis. Eligible patients were assigned to no systemic treatment (control); 2.5 mg of levamisole on 2 consecutive days each week for 48 weeks; 12 cycles of C 130 mg/m^2 orally days 1 through 14 every 4 weeks; or 12 cycles of CMF (C 80 mg/m^2 orally on days 1 through 14, methotrexate 30 mg/m^2 intravenously days 1 and 8, and 5-fluorouracil 500 mg/m^2 intravenously days 1 and 8) every 4 weeks. Endocrine treatment was not permitted. Radiotherapy to the chest wall and regional lymph nodes was given concomitant with chemotherapy (11). The levamisole arm was closed in December 1979 and the control arm in January 1981. The primary endpoint was 10-year invasive disease-free survival (DFS); events were defined as invasive locoregional recurrence, distant metastases, contralateral invasive breast cancer, second primary non–breast invasive cancer, or death irrespective of cause. Previous analyses demonstrated almost equivalent results between the C and CMF arms, as well as between the control and levamisole arms, for the primary endpoint (11). Updated information on date of death was retrieved from the Danish Central Population Registry using the civil person registration number, including follow-up until March 2015. Overall survival (OS) was calculated as the elapsed time from the date of randomization until death from any cause.

Collection of specimens, TMA construction, and immunohistochemical classification

The Biomedical Research Ethics Committee of the Capital Region of Denmark approved this translational study (KF 01-219/04, H-15012740). Formalin-fixed, paraffin-embedded blocks from primary excisional surgery specimens from patients enrolled in DBCG77B were retrospectively collected from pathology department archives throughout Denmark. Cases were selected on the basis of tumor block and tissue availability. TMAs were constructed manually using a TMA builder (Beecher Instrument ATA-27). In brief, invasive tumor areas in each donor sample were identified on hematoxylin and eosin stained sections by pathologists, and two 2.0-mm cores from representative tumor areas were transferred to recipient TMA blocks as described previously (17). Immunohistochemistry, scoring, and intrinsic subtype classification followed methods reported by Prat and colleagues (4). Six consecutive 4-μm sections from each TMA block were cut and promptly stained using the following antibodies: ER (Clone SP1, Thermo scientific), PR (Clone 1E2, Ventana), HER2 (Clone SP3, Abcam), Ki67 (Clone MIB-1, Dako), EGFR (Clone EP22, Epitomics), cytokeratin 5 (Clone XM26, Abcam) on Benchmark XT and Ultra platforms (Ventana Medical Systems). Markers were scored using published criteria by pathologists blinded to clinical outcome data. Ki67 used a scoring method analytically validated for use on TMAs (4, 18, 19). Intrinsic subtypes were defined as follows: Luminal A = hormone receptor–positive (i.e., ER and/or PR > 1%)/HER2-negative with PR > 20% and Ki67 < 14%; Luminal B = hormone receptor–positive and (PR $\leq$ 20% or HER2$^+$ or Ki67 $\geq$ 14%); HER2E = ER$^-$ and PR$^-$ and HER2$^+$), Core basal = ER/PR/HER2 triple-negative and (CK5$^+$ or EGFR$^+$).

Statistical analysis

Follow-up was quantified in terms of a Kaplan–Meier estimate. For multivariate analysis of patients treated according to protocol, a Cox proportional hazards regression model was applied to assess the adjusted HR of treatment regimen [chemotherapy (C + CMF arms) vs. no chemotherapy (control + levamisole arms)] by subtype and to explore interactions. Factors included in the multivariate analyses were age at entry ($\leq$40, 41–45, 46–50, and 51–59), tumor size (0–2, >2–5, and >5 cm), lymph node status (0–3, 4-9, and >9 positive nodes combined with 0–9 or >9 lymph

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nodes examined), histologic type, and grade (ductal grade 1 and unknowns, ductal grade 2, ductal grade 3, and other histologic types), treatment regimen and subtype. Proportional hazards assumptions were assessed using Schoenfeld residuals and by including a time-dependent component for each covariate. The hazard rates for histologic type and grade were not proportional; therefore, stratification was used. To comply with proportional hazards assumptions regarding subtypes and treatment, separate estimates were included according to time since randomization. The Wald test was used to assess heterogeneity. An exploratory analysis investigated heterogeneity of treatment effect according to amenorrhea, defined as absence of menstrual bleeding for $\geq$3 months in the first year and included as a time-dependent variable. Associations between in- and excluded patients and clinicopathologic characteristics (excluding unknowns) were analyzed using χ^2 or Fisher exact tests. *P* values are 2-tailed, unadjusted for number of comparisons. Central review, monitoring, and statistical analyses were done by the DBCG Statistical Office using the SAS 9.4 software program package (SAS Institute).

The prespecified primary hypothesis, agreed to in a written MTA between the Vancouver and DBCG groups, was that there would be an interaction (Wald heterogeneity test) between Luminal A status and chemotherapy, for the trial's original primary endpoint: 10-year DFS. Luminal A was defined as per Prat and colleagues (4) as ER-positive, PR > 20%, HER2-negative, and Ki67 < 14%, with all other cases considered non–luminal A. The following specific secondary analyses were prespecified: (i) use of OS as an alternative endpoint and (b) with reduced power, separate predictive analyses of the different non–luminal A subtypes (i.e., Luminal B, HER2E, and basal-like). Other analyses were considered exploratory.

Results

DBCG study set characteristics

The DBCG77B trial enrolled 1,146 women. From the subset treated per protocol, blocks could be obtained from about 2 of 3 of patients for inclusion in TMAs, and informative immunohistochemical results allowing unequivocal intrinsic subtype assignment were available on 633 (Fig. 1) among whom 26% had Luminal A tumors. The final study set was representative of the original trial (Table 1): mostly younger, node-positive women with large, high-grade infiltrating ductal carcinomas. Because of the early closure of the non-chemotherapy arms, 77% of women received C or CMF adjuvant chemotherapy. The study set demonstrated a benefit from chemotherapy at a level similar to that seen in the original trial.

Analysis of primary hypothesis: value of chemotherapy in Luminal A versus non–Luminal A breast cancers

In the study set of 633 patients (median estimated potential follow-up, 10 years), 318 events were observed for DBCG77B's primary endpoint of DFS. As expected, women with Luminal A tumors had better prognosis [165 patients, 61 DFS events, 5-year DFS, 74% (66–80), 10-year DFS, 61% (53–68)] than those with non–Luminal A tumor types [468 patients, 257 DFS events, 5-year DFS, 54% (49–58), 10-year DFS, 44% (39–48); Fig. 2]. The HR for benefit from chemotherapy was substantial among non–Luminal A patients (HR, 0.50). In contrast, Luminal A patients showed no apparent survival benefit from randomization to cyclophosphamide-based chemotherapy (HR, 1.06; Fig. 3). The interaction test (Wald test of heterogeneity, Luminal A status $\times$ chemotherapy) was significant, $P = 0.048$.

Prespecified secondary analyses

The primary analysis was repeated for the alternative endpoint of OS. With a median estimated potential follow-up of 34 years, a total of 497 events (deaths) occurred among the 633 patients in the study sets. Although the same trends were observed, with the non–luminal A group of patients having particularly poor outcomes when randomized to no chemotherapy, OS curves as expected trend to convergence with long term follow-up, rendering differences no longer statistically significant by log-rank test (Supplementary Fig. S1).

With an expectation of reduced power, the primary analysis was also repeated breaking the non–Luminal A group into its

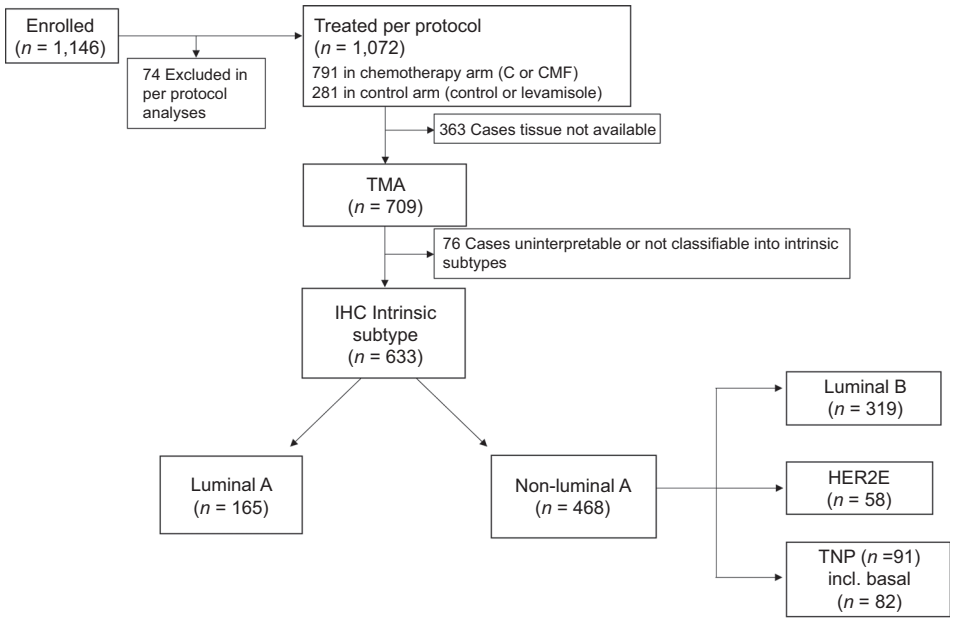

Figure 1.
CONSORT diagram.

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Table 1. Patient characteristics in the DBCG77B original trial versus the study set for which intrinsic subtype could be assigned by immunohistochemistry

	Original trial ($N = 1,072$)	Study set ($n = 633$)	Luminal A ($n = 165$)	Non–Luminal A ($n = 468$)
Age, y				
<50	741 (69%)	445 (70%)	114 (69%)	331 (71%)
≥50	331 (31%)	188 (30%)	51 (31%)	137 (29%)
Tumor size, cm				
≤2	270 (25%)	166 (26%)	51 (31%)	115 (24%)
>2	548 (51%)	353 (56%)	92 (56%)	261 (56%)
Unknown	254 (24%)	114 (18%)	22 (13%)	92 (20%)
No. of positive lymph nodes				
0	183 (17%)	90 (14%)	26 (16%)	64 (14%)
1+	889 (83%)	543 (86%)	139 (84%)	404 (86%)
Malignancy grade				
1	195 (21%)	104 (19%)	43(30%)	61 (15%)
2–3	735 (78%)	449 (81%)	98 (70%)	351 (85%)
Unknown	8 (1%)	—	—	—
Histologic type				
Ductal	938 (88%)	553 (87%)	141 (85%)	412 (88%)
Other	134 (12%)	80 (13%)	24 (15%)	56 (12%)
Chemotherapy				
Control or levamisole	281 (26%)	145 (23%)	31 (19%)	114 (24%)
C or CMF	791 (74%)	488 (77%)	134 (81%)	354 (76%)
HR (95%CI)	0.63 (0.49–0.81)	0.56 (0.43–0.72)	1.06 (0.53–2.14)	0.50 (0.38–0.66)

constituent intrinsic subtypes: Luminal B (ER-positive cases which are $HER2^+$, or Ki67 high, or PR low); basal-like (using a core basal definition of ER/PR/HER2 triple-negative + either cytokeratin 5^+ or $EGFR^+$; ref. 19); and HER2E (defined as ER^-, PR^-, and $HER2^+$). Results are presented as Forest plots in Fig. 2C and Kaplan–Meier plots in Fig. 3. Luminal B patients comprised the largest group (319 patients, 172 events) and received a significant benefit from chemotherapy [HR, 0.48; 95% confidence interval (CI), 0.35–0.67]. The basal-like group (82 patients, 37 events) also had a major benefit from cyclophosphamide-based chemotherapy (HR, 0.45; 95% CI, 0.20–0.99); exploratory analysis of triple-negatives was similar (91 patients, 42 events; HR, 0.47; 95% CI, 0.23–0.96). The HER2E group (58 patients, 43 events) all had very poor outcomes in this study, regardless of whether or not they received C/CMF chemotherapy.

Exploratory analyses

Progesterone receptor was the most recently added biomarker in the immunohistochemical definition of Luminal A breast cancer, as optimized by comparison to a gene expression profile gold standard.[4] However, although its measurement is standard in the clinical workup of breast cancer, PR is not always captured in a quantitative fashion that can readily be incorporated into a clinical definition of Luminal A. For this reason, an exploratory analysis was performed using a wider definition of Luminal A with no requirement for quantitative PR [any ER or PR+ (>1%), $HER2^-$, Ki67 < 14%]. This definition resulted in 237 patients (103 events) being classified as Luminal A, versus 396 patients (213 events) as non–luminal A. With this wider definition of Luminal A, HRs were not different between groups and there was no positive interaction with chemotherapy.

Chemotherapy-related amenorrhea, defined as absence of menstrual bleeding for at least 3 months in the first year, was recorded prospectively in DBCG77B (20). Patients did not receive endocrine therapy, and analysis of the connection between chemotherapy, amenorrhea, and DFS was added as an exploratory analysis after reviewing primary results. However, amenorrhea was not a statistical significant factor for DFS and furthermore had no heterogeneity according to ER status or Luminal A/B subtype (Supplementary Table S1). Furthermore, no significant interaction was identified between onset of amenorrhea and provision of chemotherapy, neither within the study set as a whole nor within the Luminal A subgroup.

Discussion

This formal prospective–retrospective study revealed a significant interaction between breast cancer subtype and randomization to chemotherapy, finding that Luminal A patients derive no benefit—even in a high-risk premenopausal population.

This finding is particularly important, as the original DBCG77B study demonstrated a clear benefit from adjuvant chemotherapy and left no doubt about its value among premenopausal patients with T3 or node-positive breast cancer. At 10 years, more than two thirds of control group patients experienced a DFS event despite effective locoregional treatment (11). Although we were unable to demonstrate a significant heterogeneity in Luminal A patients according to chemotherapy-related amenorrhea, the study is not powered to provide a definitive answer. Today's premenopausal patients with ER-positive and node-positive breast cancers will benefit from extended treatment with tamoxifen or a sequence of tamoxifen and an aromatase inhibitor. Endocrine treatment was not available for patients in the DBCG77B trial. The lack of endocrine therapy and its benefits left more potential room to identify any absolute benefit of chemotherapy, through direct cytotoxicity or through chemotherapy-induced amenorrhea. Nevertheless, no benefit could be demonstrated among Luminal A patients.

Strengths of study design included use of materials from a phase III trial, following best practices as defined by ReMARK guidelines and the criteria of Simon and colleagues (15, 16). The intrinsic subtype classifier (determined by immunohistochemistry on TMAs) was previously developed on independent material, with methods and results on other patient series already published (19). A formal primary hypothesis and short list of predefined secondary hypotheses was agreed to in writing prior to

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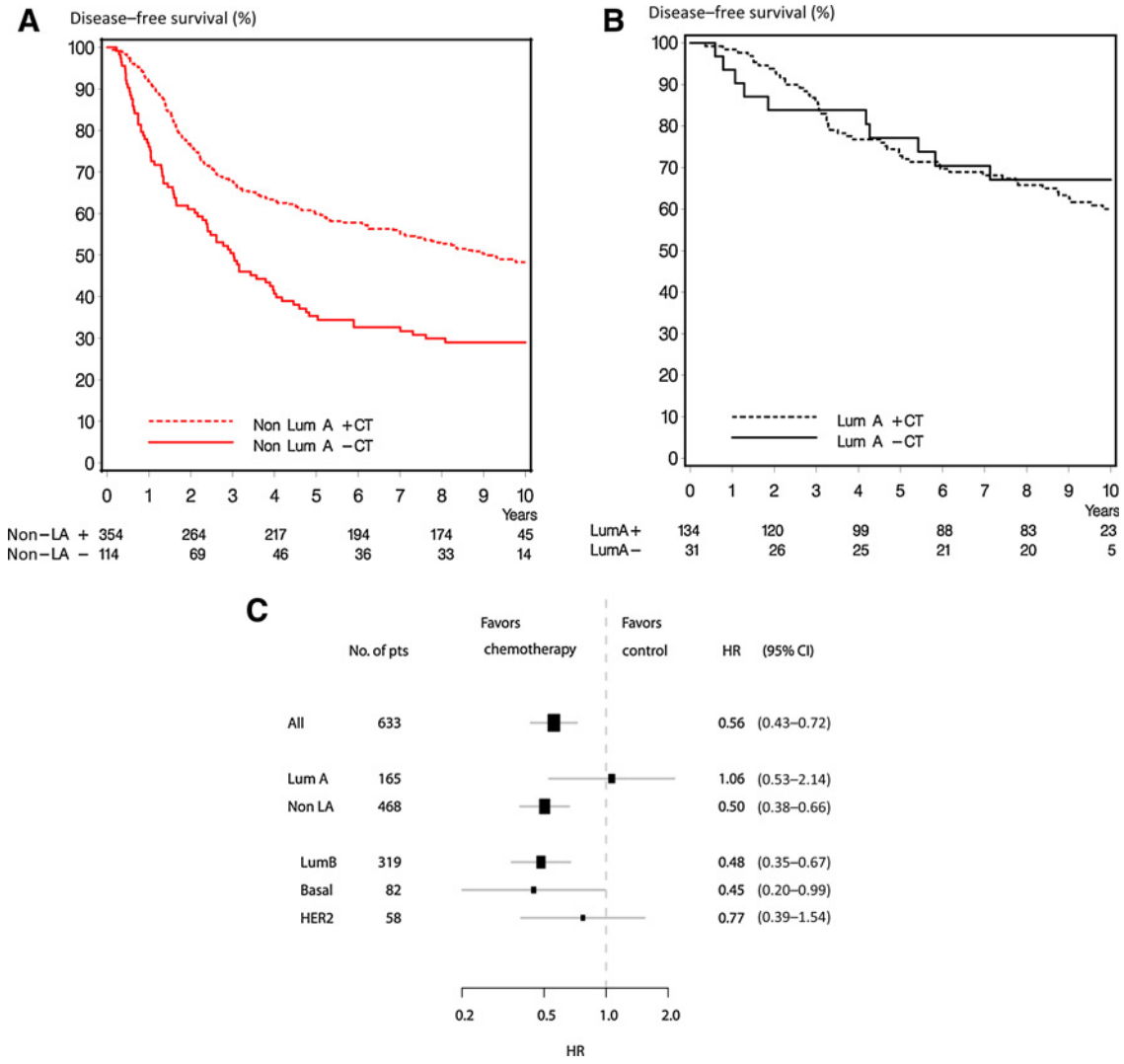

Figure 2.

Primary study endpoint: Kaplan–Meier estimates of invasive DFS for patients receiving chemotherapy (+CT, dotted lines) or randomized to non-chemotherapy arms (−CT, solid lines). **A,** Women with non–Luminal A tumor types. **B,** Women with Luminal A tumors (defined as $ER^+/HER2^-$, Ki67 < 14%, and PR > 20%). **C,** Forest plot, for the trial's original primary endpoint of 10-year DFS. Interaction test (Luminal A × Chemotherapy) $P = 0.048$.

data analysis; pathologists scoring biomarkers in Vancouver had no access to clinical data, and the prespecified statistical plan was independently executed by DBCG statisticians.

This study design yields level 2 evidence and has several limitations. Although the interaction test reached statistical significance for the primary endpoint, the critical finding that Luminal A patients derive no benefit from chemotherapy is based on comparing 134 patients receiving chemotherapy versus 31 who did not. These numbers are unavoidably limited because (i) DBCG77B enrolled clinically high-risk, younger women, a group with a low proportion of Luminal A tumors; (ii) block collection, done retrospectively long after the initial trial, was unavoidably incomplete; and (iii) the non-chemotherapy arms were closed early by the trial's data monitoring committee due to their inferiority across the trial population (not stratified by biomarkers at the time).

Furthermore, DBCG77B employed cyclophosphamide monotherapy or classic CMF chemotherapy; results therefore cannot rule out some benefit for Luminal A patients given regimens incorporating anthracyclines and taxanes. This may explain the lack of significant benefit from chemotherapy in our secondary analysis of the HER2E subset, a group expected to respond well to these drugs (trastuzumab was not an option at the time of this trial). In contrast to the HER2 subset, patients with Luminal B and basal-like tumors obtained substantial benefit from the cyclophosphamide-based chemotherapy given in DBCG77B. Several large trials support adjuvant cyclophosphamide as a highly active agent; its use is retained in most chemotherapy regimens. In contrast, controversy remains regarding the additional benefit obtainable by substituting cyclophosphamide with taxanes or adding anthracyclines if trastuzumab is provided in the HER2 group (19, 21–25).

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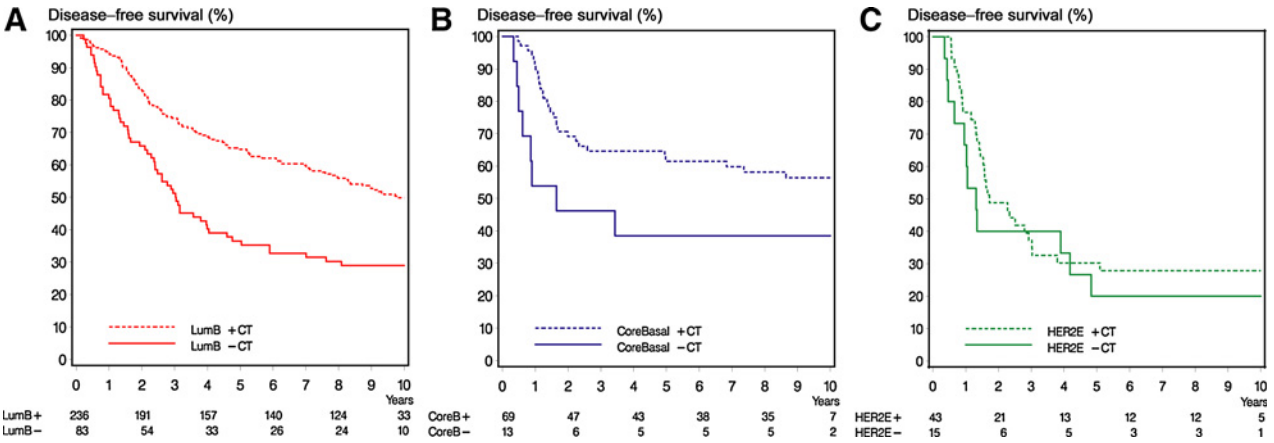

Figure 3.

Chemotherapy (CT) benefit among the 3 major non–Luminal A intrinsic subtypes of breast cancer. **A,** Luminal B. **B,** Basal-like. **C,** HER2E.

Another limitation of our study was the necessity to determine intrinsic subtype by immunohistochemistry, as the only available material was TMAs. Compared to genomics-based nucleic acid tests, immunohistochemical surrogate panels do not provide as much prognostic information (26, 27), and do not have the same level of analytical reproducibility. Although ER, PR (28), and HER2 (29) used standardized methodology (apart from preanalytical handling guidelines, not guaranteed on older specimens), Ki67 (18) and PR (30) immunohistochemistry analyses have known issues with analytical variability, particularly for quantitative analysis. These factors limit direct extrapolation of our findings into predictive tests on incident clinical specimens. Inclusion of a quantitative PR criterion appears critical, as an exploratory analysis excluding the PR > 20% criterion, resulting in a wider definition of luminal A, lost predictive significance. Nevertheless, immunohistochemistry is more widely available and less expensive than gene expression profiling for subtyping and risk stratification.

Gene expression signatures of breast cancer risk incorporating quantitative ER, PR, HER2, and Ki67 include the Recurrence Score (OncoType Dx), 70-gene signature (Mammaprint), and PAM50 (Prosigna) tests; the latter 2 have had analytical validity cleared by FDA and CE (31) and are capable of assigning Luminal A subtype. While these methods, or other expression profile tests identifying a low-risk group (32, 33), may provide a potentially more reproducible clinical test for identifying chemotherapy-insensitive patients, their application to DBCG77B would require recollection of the source blocks. As fewer are available than were during TMA construction, there will be limited power to reproduce these findings using any gene expression test, particularly considering the borderline significance of the observed interaction despite the wide HR difference.

Fully prospective studies evaluating the Recurrence Score (TailoRx) and 70-gene signature (MindACT) have to date published only prognostic, not predictive, information (34, 35); our finding that chemotherapy does not benefit even clinically high-risk luminal A patients suggests that these trials will likely confirm that chemotherapy does not benefit the lower risk, endocrine-treated populations they recruited. The Recurrence Score has been applied to NSABP-20, a trial randomizing ER-positive, node-negative women receiving tamoxifen to adjuvant CMF or no chemotherapy, and reported to be predictive (10), although this result has been criticized because the original Recurrence Score algorithm was trained on NSABP-20 (36). A subsequent prospective–retrospective study on SWOG-8814 did provide evidence for the predictive capacity of the Recurrence Score for cyclophosphamide–doxorubicin–fluorouracil among ER-positive, node-positive postmenopausal patients with breast cancer treated with tamoxifen (37). Our study of DBCG77B similarly finds that a molecular low-risk classifier (Luminal A subtype) predicts lack of benefit from chemotherapy among node-positive patients but extends this finding to premenopausal women, who did not receive adjuvant endocrine therapy. Breast cancer outcomes have markedly improved since the 1970s, to the point where cure rates now exceed 80% for early breast cancer. While on its own, our study constitutes level 2 evidence (needing confirmation on a second, similar trial), it does add to an emerging, consistent literature supporting the concept that adjuvant therapy may not confer meaningful benefit to many low-risk, Luminal A–type breast cancers (38).

Disclosure of Potential Conflicts of Interest

T.O. Nielsen has ownership interests (including patents) at Bioclassified LLC, and is a consultant/advisory board member for and reports receiving commercial research grants from NanoString Technologies. B. Ejlertsen reports receiving commercial research grants from NanoString, Novartis and Roche. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: T.O. Nielsen, M-B. Jensen, B. Ejlertsen

Development of methodology: T.O. Nielsen

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T.O. Nielsen, S. Burugu, D. Gao, C.L.T. Jørgensen, E. Balslev, B. Ejlertsen

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T.O. Nielsen, M-B. Jensen, S. Burugu, D. Gao, B. Ejlertsen

Writing, review, and/or revision of the manuscript: T.O. Nielsen, M-B. Jensen, S. Burugu, C.L.T. Jørgensen, E. Balslev, B. Ejlertsen

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T.O. Nielsen, M-B. Jensen, E. Balslev, B. Ejlertsen

Study supervision: T.O. Nielsen, B. Ejlertsen

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