Cyclin D1 Expression in Breast Cancer Patients Receiving Adjuvant Tamoxifen-Based Therapy

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Abstract

Purpose: The objective of our study was to determine the clinical relevance of cyclin D1 expression in hormone receptor – positive breast cancer patients who were treated with tamoxifen-based therapy.

Experimental Design: We assessed expression of cyclin D1 in surgical specimens of breast carcinoma by means of immunohistochemistry. Patients had been enrolled in either Austrian Breast and Colorectal Cancer Study Group (ABCSG) Trial 05 or ABCSG Trial 06 and received tamoxifen as part of their adjuvant treatment. Overall survival and relapse-free survival were analyzed with Cox models adjusted for clinical and pathologic factors.

Results: Cyclin D1 was expressed in 140 of 253 (55%) tumors of ABCSG Trial 05 and in 569 of 948 (60%) tumors of ABCSG Trial 06. Expression of cyclin D1 was associated with poor outcome in both cohorts. Overall survival was significantly shorter in patients with cyclin D1 – positive tumors compared with patients with cyclin D1 – negative tumors [adjusted hazard ratio (HR) for death (ABCSG Trial 05), 2.47; 95% confidence interval (95% CI), 1.08-5.63; $P = 0.03$; adjusted HR for death (ABCSG Trial 06), 1.78; 95% CI, 1.36-2.34; $P < 0.0001$]. Relapse-free survival was also shorter in patients with cyclin D1 – positive tumors than in patients with cyclin D1 – negative tumors [adjusted HR for relapse (ABCSG Trial 05), 2.73; 95% CI, 1.50-4.96; $P = 0.001$; adjusted HR for relapse (ABCSG Trial 06), 1.52; 95% CI, 1.14-2.04; $P = 0.005$].

Conclusion: Cyclin D1 expression is an independent poor prognostic factor in women with early-stage, hormone receptor – positive breast cancer who received adjuvant tamoxifen-based therapy.

Endocrine therapy is the most effective treatment for women with hormone receptor – positive breast cancer. For more than 20 years, the antiestrogen tamoxifen has been the established standard of care in adjuvant endocrine therapy. In the adjuvant treatment of endocrine-responsive breast cancer, 5 years of tamoxifen almost halves the annual recurrence rate and reduces the annual breast cancer death rate by a third (1). Although aromatase inhibitors have recently been shown to be even more effective, tamoxifen remains an important part of the endocrine treatment armamentarium and is still the only option in many areas around the world (2 – 7). Tamoxifen therapy is effective in many patients but *de novo* and acquired resistance remains a major problem (8). A considerable fraction of patients do not respond to tamoxifen despite having estrogen receptor – positive tumors. These patients may need other therapeutic interventions. Therefore, the ability to predict outcome of tamoxifen treatment should significantly improve the management of early-stage breast cancer.

Biomarkers have become of interest as potential predictors for outcome of adjuvant tamoxifen therapy (9). Of particular interest are biomarkers that are involved in cell cycle regulation. Cyclins, their associated cyclin-dependent kinases, and cyclin-dependent kinase inhibitory proteins play a central role in cell cycle progression and may also affect response to tamoxifen (10). One potential candidate biomarker is cyclin D1 (11). Besides the central role in cell cycle regulation, cyclin D1 directly affects the estrogen receptor and may be involved in response to estrogens and antiestrogens. Cyclin D1 has been shown to bind to the estrogen receptor and to activate the receptor in a ligand-independent fashion (12, 13). *In vitro* studies have linked tamoxifen resistance to the expression of cyclin D1 in cell lines (14 – 16). Overexpression of cyclin D1 is

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observed in ~50% of breast cancer specimens and the corresponding *CCND1* gene is amplified in 15% (17, 18). In several clinical studies, early relapse and shorter survival were observed in women with cyclin D1–positive breast cancer who received tamoxifen treatment (19–22). The purpose of our study was to determine the clinical relevance of cyclin D1 in early-stage, hormone receptor–positive breast cancer patients who had been enrolled into two randomized clinical trials and treated with adjuvant tamoxifen-based therapy.

Materials and Methods

Patients. The present investigation is part of the Austrian Breast and Colorectal Cancer Study Group (ABCSG) translational research program (abcsg.research). All patients had participated in either ABCSG Trial 05 or ABCSG Trial 06 and have received tamoxifen as part of their adjuvant treatment. Inclusion criteria and the main clinical results of both studies have been reported previously (23, 24).

The objective of ABCSG Trial 05 was to compare the efficacy of 5 years of tamoxifen (Nolvadex, AstraZeneca Pharmaceuticals) plus 3 years of goserelin (Zoladex, AstraZeneca Pharmaceuticals) with standard cyclophosphamide, methotrexate, and 5-fluorouracil chemotherapy in 1,034 premenopausal, hormone receptor–positive breast cancer patients with stage I or II disease.

The purpose of ABCSG Trial 06 was to determine whether addition of aminoglutethimide to tamoxifen improves outcome of postmenopausal patients with hormone receptor–positive, early-stage breast cancer. In brief, a total of 2,021 postmenopausal breast cancer patients were randomly assigned to receive either tamoxifen for 5 years alone or tamoxifen in combination with aminoglutethimide for the first 2 years of treatment. The primary end points in both ABCSG Trial 05 and ABCSG Trial 06 were relapse-free survival and overall survival.

Specimen collection. For the present research project, pathologists from participating ABCSG centers were asked to submit a representative formalin-fixed, paraffin-embedded tumor block from each woman to the central lab of abcsg.research at the Medical University of Vienna. Nineteen centers contributed 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 H&E to confirm the presence of invasive carcinoma histologically and further sections were used for immunohistochemical analyses. The results were reported to the ABCSG central office, where the statistical analysis was done.

Immunostaining for cyclin D1. Immunohistochemistry was done and evaluated in the abcsg.research central lab at the Medical University of Vienna by means of a standard protocol.

Briefly, tissue sections were deparaffinized and rehydrated. After heating for 10 min in 10 mmol/L citrate buffer (pH 6.0) in a pressure cooker for epitope retrieval, the tissues were incubated for 30 min at room temperature with a rabbit monoclonal antibody specific for cyclin D1 (clone SP4, 1:100 dilution; NeoMarkers). Antibody binding was detected by means of the UltraVision LP detection system according to the manufacturer's recommendations (Lab Vision Corp.). Color development was done with 3,3′-diaminobenzidine and counterstained with hematoxylin. Sections of breast carcinoma specimens known to express cyclin D1 served as external positive controls.

Cyclin D1 immunostaining was evaluated by two investigators (M.R. and M.L.) who were unaware of the patients' clinical data. All invasive tumor cells on each slide were evaluated. Interpretation of the results was limited to the invasive part of the tumor and only nuclear staining was scored as positive. The results were expressed as the percentage of cyclin D1–stained nuclei. Discordant cases were reassessed together by both investigators using a double-headed microscope until a consensus was reached. The median value of the percentage of cyclin D1–positive tumor cells in ABCSG Trial 05 was prospectively chosen as cutoff point to classify cyclin D1–positive and cyclin D1–negative tumors. This cutoff point (10% cyclin D1–positive tumor cells) was established in ABCSG Trial 05 and later used and validated in ABCSG Trial 06.

Statistical analyses. As in the clinical studies, the primary end points of these response prediction analyses were relapse-free survival and overall survival. To identify any selection bias, the baseline characteristics of patients with or without tumor blocks were compared using the χ^2 test and the survival rates were compared with the use of a Cox model. Baseline data according to cyclin D1 status were compared in univariate analyses with the use of the χ^2 test and in a multivariate logistic model adjusted for age, tumor size, lymph node status, tumor grade, estrogen receptor status, and progesterone receptor status. Survival rates were estimated with the use of the Kaplan-Meier method. The prognostic value of cyclin D1 was studied with Cox models, which were adjusted for age, tumor size, lymph node status, tumor grade, estrogen receptor, and progesterone receptor. All reported *P* values are two sided. All analyses were done with the use of Statistical Package for the Social Sciences software, version 12.0 (SPSS).

Results

ABCSG Trial 05. Ten centers provided at least one tumor block for 260 of the 511 patients who had been enrolled in the endocrine treatment arm of ABCSG Trial 05 and thus had

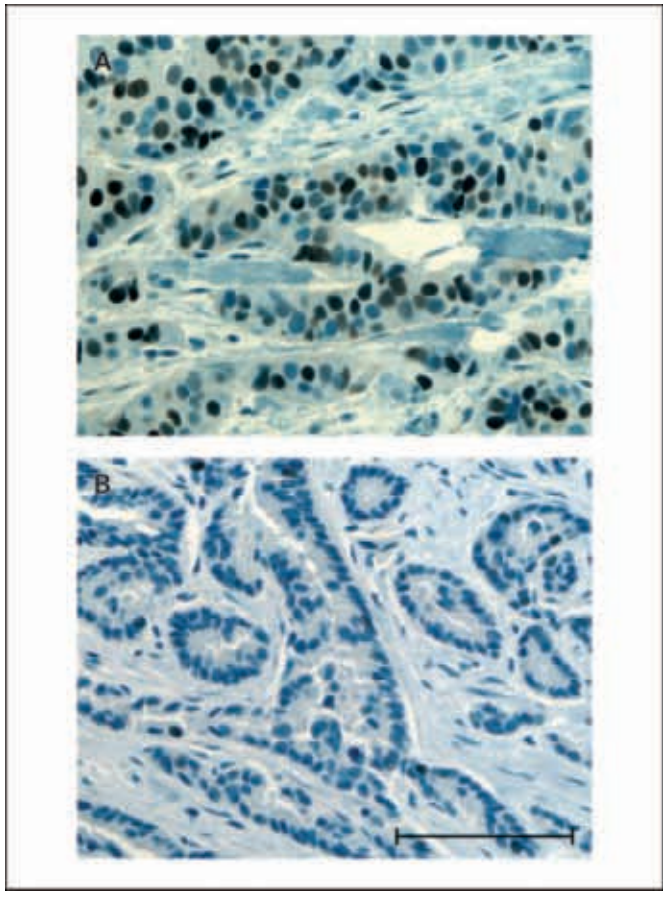

Fig. 1. Examples of cyclin D1 immunostaining. Comparison of a cyclin D1 – positive (*A*) and a cyclin D1 – negative invasive ductal breast carcinoma (*B*). The cyclin D1 – negative tumor (*B*) shows only a few positive nuclei. Bar, 100 μm.

Table 1. Characteristics of the patients enrolled in ABCSG Trial 05

Characteristic	All patients (N = 253), n (%)	Patients with cyclin D1 – negative tumors (n = 113), n (%)	Patients with cyclin D1 – positive tumors (n = 140), n (%)	P*
Age (y)				0.55
<35	16 (6)	6 (5)	10 (7)	
≥35	237 (94)	107 (95)	130 (93)	
Tumor size (cm)				0.39
T_1 (≤2)	146 (58)	61 (54)	85 (61)	
T_2 (>2 to ≤5)	96 (38)	48 (43)	48 (34)	
T_3 (>5)	11 (4)	4 (3)	7 (5)	
Affected lymph nodes				0.14
None	142 (56)	71 (63)	71 (51)	
1-3 nodes	77 (30)	32 (28)	45 (32)	
4-10 nodes	32 (13)	9 (8)	23 (16)	
>10 nodes	2 (1)	1 (1)	1 (1)	
Tumor grade				0.09
G_1, G_2	172 (68)	83 (74)	89 (64)	
G_3	81 (32)	30 (26)	51 (36)	
Estrogen receptor				0.23
Negative	19 (8)	11 (10)	8 (6)	
Positive	234 (92)	102 (90)	132 (94)	
Progesterone receptor				0.11
Negative	24 (10)	7 (6)	17 (12)	
Positive	229 (90)	106 (94)	123 (88)	
HER2 (n = 229)				0.08
Negative	202 (88)	96 (92)	106 (85)	
Positive	27 (12)	8 (8)	19 (15)	

NOTE: Percentages may not total 100 because of rounding.
*P values were calculated with the χ^2 test.

received 5 years of tamoxifen. These 260 patients and the remaining 251 had similar baseline characteristics and overall rates of survival. Among the 260 blocks, seven contained no invasive tumor and were excluded from our study. Subsequently, cyclin D1 expression was evaluated in 253 patients with early-stage, hormone receptor – positive breast cancer and further statistical analyses were done on this population.

We assessed cyclin D1 expression using standard immunohistochemistry. Cyclin D1 immunostaining was nuclear and ranged from 0% to 80% of the breast cancer cells. The median value of cyclin D1 expression of the series was 10%. Figure 1 shows representative examples of cyclin D1 immunohistochemistry. Comparisons of cyclin D1 expression with clinical variables including survival of the patients were done with cyclin D1 expression as a continuous variable and as a dichotomized variable classified as positive (>10% cyclin D1 – positive tumor cells) or negative (≤10% cyclin D1 – positive tumor cells). Of the 253 tumors, 140 (55%) were cyclin D1 positive. Table 1 compares the characteristics of the patients according to cyclin D1 expression in a univariate analysis. A multivariate logistic regression model showed that cyclin D1 expression was significantly associated with a higher level of affected lymph nodes [odds ratio, 1.53; 95% confidence interval (95% CI), 1.06-2.22; P = 0.02].

The survival data reported here are based on an updated data set from ABCSG Trial 05. With a median follow-up of 11 years, 63 of 253 (25%) patients relapsed (48 patients with cyclin D1 – positive tumors and 15 patients with cyclin D1 – negative tumors; P < 0.0001) and 34 of 253 (13%) patients had died (26 patients with cyclin D1 – positive tumors and 8 patients with cyclin D1 – negative tumors; P = 0.008). Age [hazard ratio (HR) for relapse, 0.27; 95% CI, 0.14-0.52; P < 0.0001], tumor size (HR for relapse, 1.89; 95% CI, 1.25-2.86; P = 0.002), lymph node status (HR for relapse, 1.69; 95% CI, 1.28-2.24; P < 0.0001), tumor grade (HR for relapse, 2.05; 95% CI, 1.27-3.31; P = 0.004), and cyclin D1 (HR for relapse, 3.08; 95% CI, 1.72-5.50; P < 0.0001) were significantly associated with relapse-free survival in the univariate analyses (Fig. 2). Tumor grade (HR for death, 2.89; 95% CI, 1.52-5.48; P = 0.001), progesterone receptor status (HR for death, 0.33; 95% CI, 0.15-0.72; P = 0.005), and cyclin D1 (HR for death, 2.97; 95% CI, 1.34-6.55; P = 0.007) were significantly associated with overall survival in the univariate analyses as well (Fig. 2). Similar results were obtained if cyclin D1 was analyzed as a continuous variable (HR for relapse, 1.02; 95% CI, 1.01-1.04; P < 0.0001; HR for death, 1.02; 95% CI, 1.00-1.04; P = 0.03).

The independent effect of cyclin D1 expression on relapse-free survival and overall survival was assessed by Cox models adjusted for age, tumor size, lymph node status, tumor grade, estrogen receptor status, and progesterone receptor status. In these multivariate analyses, cyclin D1 expression was significantly associated with relapse-free survival (adjusted HR for relapse, 2.73; 95% CI, 1.50-4.96; P = 0.001) and overall survival (adjusted HR for death, 2.47; 95% CI, 1.08-5.63; P = 0.03) of the patients (Fig. 2). Comparable results for cyclin D1 were obtained if HER2 status was included into the Cox models (adjusted HR for relapse, 3.30; 95% CI, 1.75-6.22; P < 0.0001; adjusted HR for death, 3.40; 95% CI, 1.34-8.61; P = 0.01).

ABCSG Trial 06. The study group consisted of 948 postmenopausal women with early-stage, hormone receptor – positive

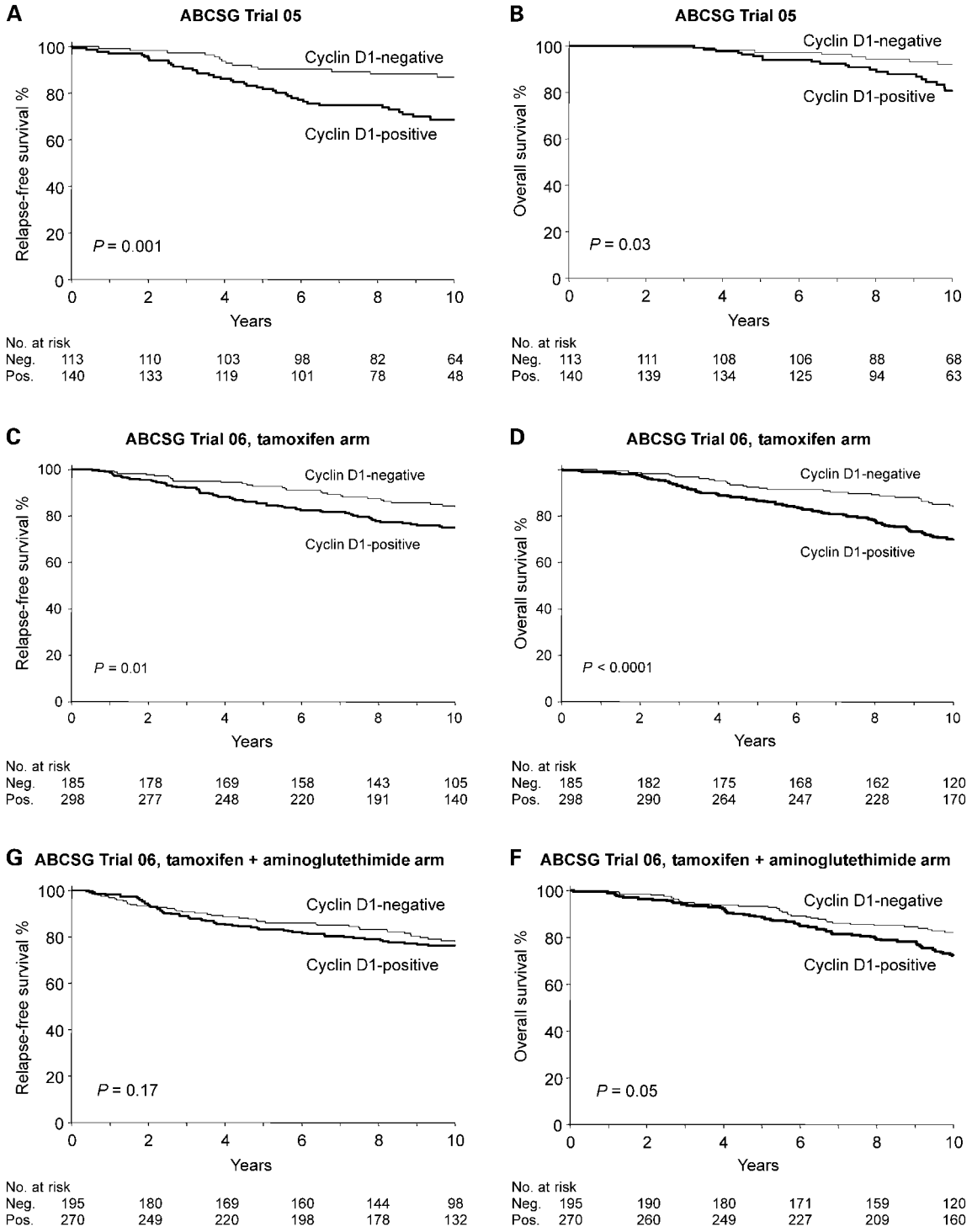

Fig. 2. Survival according to cyclin D1 status. Figure 2 shows relapse-free survival (*A, C*, and *E*) and overall survival (*B, D*, and *F*) according to cyclin D1 status of the women with breast cancer enrolled in ABCSG Trial 05 and ABCSG Trial 06.

breast cancer who had been enrolled in ABCSG Trial 06. Nineteen centers contributed at least one tumor block for each patient. The 948 patients with tumor blocks and the remaining 1,038 patients without tumor blocks had similar baseline characteristics and overall rates of survival. The median value of cyclin D1–stained nuclei was 20% (range, 0-100%). Comparisons of cyclin D1 expression with clinical variables including survival of the patients were done with the cutoff point previously defined in ABCSG Trial 05. Cyclin D1 expression was classified as positive (>10% cyclin D1–positive tumor cells) or

negative (≤10% cyclin D1–positive tumor cells). Of the 948 tumors, 569 (60%) were cyclin D1 positive. Table 2 compares the characteristics of the patients according to cyclin D1 expression in a univariate analysis. A multivariate logistic regression model showed that cyclin D1 expression was significantly associated with age (odds ratio, 1.31; 95% CI, 1.12-1.53; $P = 0.001$) and tumor size (odds ratio, 1.32; 95% CI, 1.03-1.71; $P = 0.03$).

With a median follow-up of 11 years, 210 of 948 (22%) patients had relapsed (139 patients with cyclin D1–positive tumors and 71 patients with cyclin D1–negative tumors; $P = 0.04$) and 270 of 948 (29%) patients had died (197 patients with cyclin D1–positive tumors and 73 patients with cyclin D1–negative tumors; $P < 0.0001$). Age (HR for relapse, 0.81; 95% CI, 0.69-0.95; $P = 0.009$), tumor size (HR for relapse, 1.93; 95% CI, 1.55-2.41; $P < 0.0001$), lymph node status (HR for relapse, 2.06; 95% CI, 1.80-2.35; $P < 0.0001$), tumor grade (HR for relapse, 1.52; 95% CI, 1.12-2.07; $P = 0.008$), progesterone receptor (HR for relapse, 0.62; 95% CI, 0.46-0.82; $P = 0.001$), and cyclin D1 (HR for relapse, 1.43; 95% CI, 1.07-1.90; $P = 0.02$) were significantly associated with relapse-free survival in the univariate analyses. Age (HR for death, 1.69; 95% CI, 1.45-1.97; $P < 0.0001$), tumor size (HR for death, 1.84; 95% CI, 1.51-2.23; $P < 0.0001$), lymph node status (HR for death, 1.67; 95% CI, 1.47-1.89; $P < 0.0001$), tumor grade (HR for death, 1.38; 95% CI, 1.05-1.82; $P = 0.02$), progesterone receptor (HR for death, 0.68; 95% CI, 0.53-0.89; $P = 0.004$), and cyclin D1 (HR for death, 1.93; 95% CI, 1.47-2.52; $P < 0.0001$) were significantly associated with overall survival in the univariate analyses as well.

The independent effect of cyclin D1 expression on relapse-free survival and overall survival was assessed by Cox models adjusted for age, tumor size, lymph node status, tumor grade, estrogen receptor status, progesterone receptor status, and treatment. In these multivariate analyses, cyclin D1 expression was significantly associated with relapse-free survival (adjusted HR for relapse, 1.52; 95% CI, 1.14-2.04; $P = 0.005$) and overall survival (adjusted HR for death, 1.78; 95% CI, 1.36-2.34; $P < 0.0001$) of the patients. Similar results for cyclin D1 were obtained when the subgroups of patients from the tamoxifen arm (HR for relapse, 1.77; 95% CI, 1.15-2.74; $P = 0.01$; HR for death, 2.22; 95% CI, 1.49-3.32; $P < 0.0001$) and from the tamoxifen plus aminoglutethimide arm (HR for relapse, 1.32; 95% CI, 0.89-1.96; $P = 0.17$; HR for death, 1.47; 95% CI, 1.01-2.14; $P = 0.05$) were analyzed (Fig. 2; Table 3).

Discussion

A positive hormone receptor status has been considered the standard for predicting response to endocrine therapies in breast cancer patients. However, in many cases, phenotypic expression of hormone receptors is not sufficient to ensure efficient therapeutic response because additional molecular alterations affect the sensitivity of tumor cells to endocrine treatment. A further selection of women with hormone-dependent breast cancer in addition to the hormone receptor

Table 2. Characteristics of the patients enrolled in ABCSG Trial 06

Characteristic	All patients ($N = 948$), n (%)	Patients with cyclin D1–negative tumors ($n = 379$), n (%)	Patients with cyclin D1–positive tumors ($n = 569$), n (%)	P*
Age (y)				0.007
<51	32 (3)	15 (4)	17 (3)	
51-60	282 (30)	134 (35)	148 (26)	
61-70	350 (37)	134 (35)	216 (38)	
>70	284 (30)	96 (25)	188 (33)	
Tumor size (cm)				0.01
T_1 (≤2)	571 (60)	250 (66)	321 (56)	
T_2 (>2 to ≤5)	349 (37)	119 (31)	230 (40)	
T_3 (>5)	28 (3)	10 (3)	18 (3)	
Affected lymph nodes				0.23
None	579 (61)	246 (65)	333 (59)	
1-3 nodes	253 (27)	92 (24)	161 (28)	
4-10 nodes	86 (9)	29 (8)	57 (10)	
>10 nodes	30 (3)	12 (3)	18 (3)	
Tumor grade				0.04
G_1, G_2, unknown	756 (80)	315 (83)	441 (78)	
G_3	192 (20)	64 (17)	128 (23)	
Estrogen receptor				1.00
Negative	25 (3)	10 (3)	15 (3)	
Positive	923 (97)	369 (97)	554 (97)	
Progesterone receptor				0.49
Negative	224 (24)	94 (25)	130 (23)	
Positive	724 (76)	285 (75)	439 (77)	
Adjuvant therapy				0.28
Tamoxifen	483 (51)	185 (49)	298 (52)	
Tamoxifen + aminoglutethimide	465 (49)	194 (51)	271 (48)	

NOTE: Percentages may not total 100 because of rounding.
*P values were calculated with the χ^2 test.

Table 3. Multivariable analyses with the use of Cox models

Variable	HR for relapse (95% CI)	*P*	HR for death (95% CI)	*P*
ABCSG Trial 05 (*n* = 229)				
Age	0.35 (0.17-0.74)	0.006	0.79 (0.27-2.33)	0.67
Tumor size	1.81 (1.17-2.80)	0.008	1.75 (0.99-3.10)	0.06
Lymph nodes	1.40 (1.01-1.93)	0.04	0.88 (0.54-1.42)	0.59
Tumor grade	1.51 (0.89-2.57)	0.13	2.97 (1.38-6.41)	0.006
Estrogen receptor	0.47 (0.19-1.16)	0.10	0.72 (0.16-3.20)	0.67
Progesterone receptor	0.75 (0.35-1.62)	0.46	0.44 (0.18-1.05)	0.06
HER2	0.73 (0.31-1.74)	0.48	0.89 (0.29-2.67)	0.83
Cyclin D1	3.30 (1.75-6.22)	<0.0001	3.40 (1.34-8.61)	0.01
ABCSG Trial 06, tamoxifen arm (*n* = 483)				
Age	0.86 (0.68-1.10)	0.23	1.81 (1.45-2.27)	<0.0001
Tumor size	1.61 (1.12-2.32)	0.01	1.39 (1.02-1.89)	0.04
Lymph nodes	1.96 (1.59-2.43)	<0.0001	1.56 (1.29-1.89)	<0.0001
Tumor grade	1.12 (0.70-1.79)	0.63	1.25 (0.83-1.88)	0.28
Estrogen receptor	0.67 (0.21-2.17)	0.50	0.48 (0.19-1.20)	0.12
Progesterone receptor	0.64 (0.42-0.99)	0.05	0.63 (0.44-0.91)	0.01
Cyclin D1	1.77 (1.15-2.74)	0.01	2.22 (1.49-3.32)	<0.0001
ABCSG Trial 06, tamoxifen + aminoglutethimide arm (*n* = 465)				
Age	0.72 (0.57-0.91)	0.006	1.61 (1.29-2.00)	<0.0001
Tumor size	1.42 (1.04-1.96)	0.03	1.56 (1.18-2.06)	0.002
Lymph nodes	1.94 (1.58-2.39)	<0.0001	1.65 (1.36-2.00)	<0.0001
Tumor grade	0.80 (0.50-1.28)	0.36	0.83 (0.54-1.27)	0.39
Estrogen receptor	1.51 (0.37-6.22)	0.57	3.79 (0.53-27.25)	0.19
Progesterone receptor	0.49 (0.32-0.73)	0.001	0.73 (0.49-1.08)	0.12
Cyclin D1	1.32 (0.89-1.96)	0.17	1.47 (1.01-2.14)	0.05

status for endocrine therapies using predictive biomarkers would obviously be of major clinical relevance. The cell cycle regulator cyclin D1 is one potential candidate biomarker.

In our current study, we showed that expression of cyclin D1 predicted poor outcome of breast cancer patients who had received adjuvant tamoxifen-based therapy. Women with cyclin D1–negative tumors had a significantly longer relapse-free survival and overall survival than women with cyclin D1–positive tumors.

These results are supported by *in vitro* studies, which suggest that expression of cyclin D1 is associated with resistance to tamoxifen, and by previous clinical studies, which suggested that women with hormone-dependent breast cancer and cyclin D1–positive tumors have a poor outcome after tamoxifen treatment (14–16, 19–22). However, the current analysis cannot separate the potential predictive role of cyclin D1 in the adjuvant tamoxifen setting from its prognostic effect.

Over the last 20 years, tamoxifen has been the standard of care for women with hormone receptor–positive breast cancer. Although several recent studies suggested that aromatase inhibitor therapy may be a slightly more effective endocrine strategy against hormone-dependent breast cancer, tamoxifen may still play an important role in the adjuvant endocrine treatment of these patients (3–5, 7, 25). Some patients are unsuitable for aromatase inhibitor therapy due to the side effects of the agents; others may be unsuitable due to preexisting bone problems, which many consider an aromatase inhibitor contraindication (26–29). The optimal adjuvant treatment for such patients has not yet been established. Furthermore, in many parts of the worlds, tamoxifen remains the only economically affordable treatment option for women with endocrine-responsive breast cancer for health economic reasons. In addition, endocrine intervention in low-risk breast cancer patients may need to be continued for longer periods, as we increasingly understand the biology of breast carcinoma as a chronic disease and the need for extended adjuvant intervention to prevent the late risk of relapse. This might as well constitute an argument for keeping tamoxifen in the adjuvant armamentarium as it is done in concepts of early or delayed treatment switch (3–5, 7, 25). In particular, for those women who relapse or develop metastases after aromatase inhibitor therapy, tamoxifen may be still a reasonable choice.

Gene expression profiling of breast tumor may also identify novel biomarkers predicting tamoxifen response. Two types of assays, a multiplex reverse transcription-PCR assay and a two-gene expression index (HOXB13:IL17BR), have been described to be useful for predicting outcome of patients who received tamoxifen therapy (30, 31). In postmenopausal women from a randomized adjuvant tamoxifen trial, the two-gene index was predictive of both early relapse and death in node-negative patients but not in node-positive patients (32).

This study was conducted according to a detailed working plan that stressed the importance of collecting most of the tumor specimens within the participating centers, required a large sample size to ensure adequate power for survival analyses, and specified a statistical plan of analysis. Adjusting on standard prognostic variables and specifying an objective cutoff point for defining positivity in an independent trial cohort strengthen the reported results. We applied the cutoff point determined in ABCSG Trial 05 and laboratory method used for detection of cyclin D1 expression exactly the same way as in ABCSG Trial 06. Patients from both ABCSG Trial 05 and ABCSG Trial 06 had been included in randomized clinical trials, which further corroborate our results.

In conclusion, women with hormone receptor–positive, early-stage breast cancer and cyclin D1–negative tumors have a longer survival after adjuvant tamoxifen-based therapy

compared with women with cyclin D1–positive tumors. Whether cyclin D1 expression may help to select patients who will benefit from tamoxifen from those who will not will have to be determined in additional studies.

Appendix

The following investigators and pathologists also participated in this study: B. Gebhard and D. Kandioler (Department of Surgery, Medical University of Vienna, Vienna, Austria); G. Altorjai, R. Bartsch, U. Pluschnig, G. Steger, C. Wenzel, and C. Zielinski (Department of Internal Medicine I, Medical University of Vienna); P. Mayer, C. Menzel, B. Mlineritsch, C. Rass, R. Reitsamer, and G. Russ (Third Medical Department and Department of Special Gynecology, General Hospital Salzburg, Salzburg, Austria); T. Bauernhofer, H-J. Mischinger, F. Ploner, M. Smola, P. Steindorfer, and H. Stöger (Departments of Internal Medicine and Surgery, Medical University of Graz, and Second Department of Surgery, General Hospital Graz, Graz, Austria); E. Asseryanis, R. Möslinger-Gehmayr, and M. Seifert (Division of Special Gynecology, Medical University of Vienna); D. Depisch, K. Haider, A. Lenauer, E. Markis, and T. Payrits (Departments of Pathology and Surgery, General Hospital Wiener Neustadt, Wiener Neustadt, Austria); A. Galid, R. Grill, H. Matzinger, A. Nader, and H. Spoula (Departments of Pathology-Histology, Surgery, and Gynecology, Hanusch Medical Center, Vienna, Austria); S. Taucher (Department of Gynecology, Medical University of Innsbruck, Innsbruck, Austria); M. Schmid (Department of Internal Medicine, BHB Hospital Graz, Graz, Austria); R. Kocher, G. Leitner, F. Stangl, and R. Stering (Departments of Pathology and Surgery, Leoben Hospital, Leoben, Austria); G. Luschin-Ebengreuth and R. Winter (Department of Gynecology, Medical University of Graz); E. Hanzal and C. Sam (Division of Gynecology and Obstetrics, Medical University of Vienna); P. Kier, A. Reiner-Concin, and K. Renner (Second Medical Department and Departments of Pathology and Surgery, SMZ Ost Hospital, Vieena, Austria); H. Hartleb, H. Ludwig, and P. Sagaster (First Medical Department and Department of Pathology, Wilhelminenspital, Vienna, Austria); R. Greul, G. Hochreiner, G. Syré, C. Tausch, and G. Wahl (First Medical Department and Departments of Surgery and Pathology, Linz Hospital, Linz, Austria); F. Kugler, G. Michlmayer, and R. Schildberger (Departments of Surgery and Internal Medicine, BHS Hospital, Linz, Austria); F. Hofbauer and M. Lang (Department of Surgery, Oberpullendorf Hospital, Oberpullendorf, Austria); G. Bayer-Tiefenbach and K. Mach (Departments of Surgery and Pathology, Hospital Oberwart, Oberwart, Austria); H. Feichtinger and J. Schueller (First Medical Department and Department of Pathology, Hospital Rudolfstiftung, Vienna, Austria); F. Burger and O. Braun (Departments of Gynecology and Pathology, Waldviertel Klinikum, Horn, Austria); M. Medl and W. Ulrich (Departments of Gynecology and Pathology, Lainz Hospital, Vienna, Austria); H. Brustmann, S. Naudé, and P. Riss (Departments of Gynecology and Pathology, Mödling Hospital, Mödling, Austria).

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