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Cyclin E confers a prognostic value in premenopausal breast cancer patients with tumours exhibiting an infiltrative growth pattern

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Abstract

Aim: This study investigates the prognostic value of cyclin E in relation to tumour growth pattern by analysing stage II primary breast cancers from premenopausal women not subjected to any further adjuvant treatment. In addition, the value of cyclin E as a predictor of tamoxifen response was analysed, by comparing untreated and treated patients with oestrogen receptor positive tumours.

Methods: Breast cancer samples, assembled in tissue microarrays, were immunohistochemically stained for cyclin E and evaluated regarding the presence of nuclear staining. The overall growth characteristics of each tumour were assessed using whole tissue sections.

Results: Tumours displaying a pushing margin phenotype were strongly associated with high cyclin E levels, lymph node negative disease, a high histological grade, ER negativity, and exhibited a better prognosis compared to tumours with an infiltrative growth pattern. In the total cohort of non-treated patients (N=187), cyclin E was not associated with recurrence free survival (RFS). However, when analysing the subgroup of tumours lacking a pushing growth pattern (N=141), cyclin E was significantly associated with RFS, independent of histological grade and node status. There was no significant difference in tamoxifen response with regard to different cyclin E levels.

Conclusion: The prognostic value of cyclin E in premenopausal breast cancer is limited to patients with breast carcinomas exhibiting an exclusively infiltrative growth pattern. This limitation could be explained by the presence of a small but distinct subgroup of cyclin E-high breast cancers with a pushing margin phenotype and a more favourable outcome.

Keywords: Breast cancer, cyclin E, tumour growth pattern, medullary carcinoma, tamoxifen

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Introduction

Breast cancer is the most common cancer type among women in the Western World. Clinically important prognostic factors include histological grade, lymph node status, age, and tumour size. Due to the relative high risk of recurrence, the majority of patients are offered adjuvant treatment such as chemotherapy and/or endocrine therapy. However, a proportion of these patients will be cured by the initial surgery and thus do not need additional treatment. It is therefore of great importance to discriminate between subtypes of breast tumours in order to refine the selection of patients that indeed benefit from adjuvant therapy.

Cyclin E has been extensively investigated as being one of several new candidate prognostic factors. Deregulation of cyclin E is observed in a subgroup of breast cancer and several findings suggest that cyclin E has oncogenic properties. Cyclin E is an important regulator of the G_1/S-phase transition and has in experimental settings been shown to induce an accelerated S-phase entry [1, 2]. Deregulation of cyclin E may also induce chromosome instability by triggering an inappropriate initiation of DNA replication and centrosome duplication [3-5]. Further, transgenic mice with targeted expression of cyclin E in the mammary gland suffer from a higher incidence of mammary carcinomas [6], supporting a role for cyclin E in tumorigenesis.

High cyclin E expression in breast tumours have been shown to be associated with a poor clinical outcome [7-11]. However, there are some inconsistencies between existing studies and other groups have not been able to verify cyclin E as an independent prognostic factor in breast cancer [12-14]. There may be numerous reasons for this discrepancy, such as differences in the quantification of cyclin E, heterogeneity regarding patient cohorts and administration of adjuvant therapy. Anti-oestrogen treatment is of particular importance since cyclin E deregulation is strongly correlated to ER negative disease [8].

Recently, we observed that stable overexpression of cyclin E in MDA-MB-468 breast cancer cells induced a decreased motility and invasive potential, in addition to an elevated S-phase fraction [15]. High levels of cyclin E in primary breast tumours were further shown to be associated with a low infiltrative, “pushing margin” growth pattern [15]. The presence of a pushing tumour margin has been described as a common feature of the ER negative subgroup with correlations to negative lymph node status [16]. In addition, a pushing, well-delimited growth pattern is a property that partly defines the medullary type of breast cancer, a histological type that is more prevalent among younger women and presents contradictive properties such as a high histological grade and a more favourable outcome [17]. The association between high cyclin E levels and low invasiveness/infiltrative growth seem, however, to contradict the suggested aggressive consequences of cyclin E deregulation. Speculatively, this could reflect a difference in how specific tumours are able to simultaneously combine proliferative- and invasive activities, where invasiveness is restrained by a frequent proliferation in certain but not all tumours. Overall, this could indicate that tumour growth characteristics might contribute to a more detailed understanding of how cyclin E expression is related to breast cancer prognosis.

Endocrine treatment is often an efficient therapy for ER positive breast cancer [18, 19]. However, despite the presence of ER some patients fail to respond or eventually develop resistance to treatment [20]. The reason for tamoxifen resistance is poorly understood but co-activators of the ER, such as cyclin D1 [21-23] and the epidermal growth factor receptors [24] might be involved in inhibiting tamoxifen response. The role of cyclin E in anti-oestrogen resistance is not well established, although some studies have shown, experimentally, that overexpression of cyclin E may have a negative effect on tamoxifen response [25-27]. In one report, high levels of cyclin E mRNA correlated to endocrine therapy failure [28]. However, the

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relevance of cyclin E as a predictor of tamoxifen response is rather difficult to assess, due to the low frequency of cyclin E deregulation in ER positive tumours.

In the present study, we have evaluated the significance of cyclin E and tumour growth pattern, alone and in combination, as prognostic factors for recurrence free survival (RFS) in the untreated arm of a premenopausal breast cancer study, where patients had been randomised to two years of tamoxifen or no treatment. Furthermore, we analysed the value of cyclin E in predicting tamoxifen response by comparing untreated and treated patients with ER positive tumours.

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Patients and Methods

Patient material

Between 1986 and 1991, 564 premenopausal patients with primary invasive stage II breast cancer were enrolled in a previously described randomised clinical trial of adjuvant tamoxifen treatment [29]. All patients were operated by modified radical mastectomy or breast-conserving surgery including axillary lymph node dissection. Radiotherapy was delivered to the breast after breast-conserving surgery and patients with axillary lymph node metastases received loco-regional radiotherapy. In less than 2% of the patients (n=9), adjuvant polychemotherapy or goserelin was given. The median follow-up for patients without breast cancer event was 13.9 years. In the event of a local or distant recurrence, tamoxifen was administered to receptor positive patients randomised to no adjuvant treatment at the initiation of the study. As a consequence, we have chosen to use recurrence free survival (RFS) as a measure of disease outcome in the survival analysis. The ethical committees at Linköping and Lund Universities have approved the study.

Immunohistochemistry

Formalin fixed, paraffin embedded tumour material was available in 500 cases and representative parts of the primary tumours were selected and assembled in tissue microarrays (TMAs) using an automated tissue arrayer (ATA-27, Beecher Instruments Microarray Technology, Woodland, MD, USA). Four µm sections were deparaffinized using xylen and rehydrated using graded ethanol. Slides were then microwave treated in TRS buffer pH 9.1 (Target Retrieval Solution, S3307, DAKO, Denmark) and the staining procedure was carried out with an automated immunohistochemistry-staining machine (DAKO Techmate 500, DAKO, Denmark) using the Envision program (EnVision Systems, DAKO). The antibody against cyclin E (HE-12, Santa Cruz Biotechnology, CA, USA) was diluted 1:50. Antibody reactivity was verified using Western blotting of tumour extracts and cell lines (data not shown).

Cyclin E was evaluated for the presence of nuclear staining. Cyclin E immunoreactivity was scored as the fraction of positive nuclei divided into four groups: 0-1%, 2-10%, 11-50%, 51-100%, which were then further divided into three groups: cyclin $E^{negative}$ (0-1%), cyclin E^{low} (2-10%) and cyclin E^{high} (11-100%). In addition, cyclin E nuclear intensity was scored as negative, weak, moderate or strong. Oestrogen- and progesterone receptor status had been assessed previously [29] where tumours with more than 10% positive nuclei were considered positive, in line with the current clinical guidelines.

Assessment of tumour growth pattern

Two independent investigators evaluated the overall growth characteristics of each tumour on whole sections. Growth patterns were divided into four groups defined by the mode of infiltration, ranging from tumours with an exclusively "sieving" and diffuse infiltration (90-100%, group 1) to tumours with an exclusively circumscribed growth pattern with pushing margins (90-100%, group 4). Two intermediary groups were defined, accounting for tumours with a predominantly infiltrative (50-90%) or a predominantly pushing (50-90%) growth (group 2 and 3, respectively). Cases with multifocal tumours and an obvious intertumoral heterogeneity regarding the growth pattern were omitted from the analysis.

Statistical analysis

Differences in distribution of various clinicopathological parameters in regard to cyclin E expression and growth pattern were calculated using the χ^2 test, Mann-Whitney *U* test and Kruskal-Wallis test. RFS curves were performed using the Kaplan-Meier method and the statistical significance determined by the log-rank test. Cox uni- and multivariate analysis

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were used to explore the impact of cyclin E and growth pattern on RFS. All statistical calculations were performed using SPSS version 11.0 (SPSS inc., Chicago, IL, USA).

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Results

The associations of cyclin E protein levels and tumour growth pattern with patient and tumour characteristics in the total cohort of cases (treated and untreated) are shown in table 1 and 2, respectively. In total, 385 (77%) tumours were successfully evaluated for cyclin E protein expression and in the remaining 115 (23%) cases biopsies were either missing or did not contain enough tumour cells to be evaluated. The fraction of cyclin E positive nuclei and nuclear staining intensity correlated significantly with each other (Spearman's rho, r=0.60). Cyclin E expression was strongly associated with larger tumour size and higher histological grade and further with negative steroid hormone receptor status (valid both for ER and PR). Cyclin E was also associated with a circumscribed, pushing growth pattern, as illustrated in figure 1A-B. This association was clearly reflected in the high frequency of cyclin E^{high} medullary carcinomas.

Tumour specific growth pattern was assessed in 472 (94%) cases. The majority of the tumours (N=349, 74%) were exclusively infiltrative while 13 (3%) tumours exhibited an exclusively pushing margin phenotype. The remaining tumours exhibited either a predominantly infiltrative growth (N=62, 13%) or a predominantly pushing growth (N=48, 10%). Presence of a pushing margin was clearly associated with larger tumour size, higher histological grade and negative lymph node- and hormone receptor status. The major associations between tumour growth patterns and clinicopathological characteristics in the total cohort of cases (treated and untreated) are illustrated in figure 2A. There was further an association between tumour growth pattern and histological type, such that all tubular and lobular tumours, with the exception of one lobular carcinoma with an exclusively alveolar growth pattern, were classified as exclusively infiltrative and all medullary carcinomas as predominantly or exclusively pushing.

In order to assess the prognostic value of cyclin E and tumour growth pattern without the influence of adjuvant therapy, these factors were analysed in relation to RFS in the untreated control group. We first visualised the relation between tumour growth pattern and RFS in Kaplan-Meier analysis. Pushing growth pattern features were associated with a better prognosis (figure 2B, P(trend)=0.028). We next turned to the relation between cyclin E expression and RFS. The Kaplan-Meier analysis revealed no association between cyclin E protein levels and prognosis (fig 3A, P(trend)=0.469). However, because pushing growth pattern features were more prevalent among the cyclin E^{high} tumours (table 1), we chose to examine the prognostic impact of cyclin E expression in relation to growth pattern. Figure 3A-D illustrates how the prognostic association of cyclin E expression depended on growth pattern. When focusing on the majority of tumours that exhibited an exclusively infiltrative growth pattern (figure 3D), higher cyclin E protein levels did indeed correlate to a worse RFS (P(trend)=0.005). The impact of tumour growth pattern on the prognostic value of cyclin E is further visualised in figure 4, where the supposedly aggressive cyclin E^{high} tumours exhibited a clear prognostic variation depending on their specific growth pattern (P(trend)=0.001). The result of Cox univariate and multivariate analyses are summarised in table 3 for the entire control cohort and in table 4 for the major subgroup consisting of exclusively infiltrative tumours. In the total control cohort, only histological grade, lymph node metastasis and tumour growth pattern significantly correlated to RFS. When medullary carcinomas were excluded, the prognostic value of growth pattern was only of boarder line significance (HR=0.68, 95% CI=0.46-1.02, P=0.062). In the major subgroup of exclusively infiltrative tumours, cyclin E contributed with prognostic information independently of grade and lymph node status (HR 1.40, 95% CI=1.05-1.87, P=0.02).

In order to investigate whether cyclin E is a predictor of tamoxifen response, cyclin E was analysed in patients with ER positive tumours subjected to tamoxifen therapy or no adjuvant treatment. As previously described [29], patients with ER positive tumours benefited

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from 2 years of adjuvant therapy with tamoxifen (p=0.01) in contrast to patients with ER negative tumours (p=0.77, figure 5A-B). When analysing the impact of cyclin E expression on tamoxifen response in the ER positive group, no trend could be seen in treatment response with increasing levels of cyclin E expression (figure 5C). These findings indicate that there was no significant difference in tamoxifen response with regard to cyclin E expression.

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Discussion

The aim of this study was to assess the prognostic and treatment predictive value of cyclin E in premenopausal stage II primary breast carcinomas. Although several studies have investigated possible prognostic and predictive implications of cyclin E expression in breast cancer, the results are not conclusive. One potentially important explanation for the inconsistencies between studies might be the use of mixed tumour materials with regard to both administration of adjuvant treatment and patient age. Since the level of cyclin E expression is inversely associated to ER- and PR status, the relatively more favourable outcome for patients with cyclin E low tumours is probably affected by the treatment effect of endocrine therapy. To obtain prognostic information, cyclin E needs to be analysed in patient cohorts that do not receive adjuvant treatment after surgery. Such cohorts are not obtainable today, since the vast majority of breast cancer patients receive adjuvant endocrine- and/or chemotherapy. We therefore analysed a completed randomised trial with long-term follow-up including a non-treated control group.

In our analysis of the total cohort of untreated patients (N=187), we were not able to show any association between RFS and cyclin E protein levels, contrasting other reports stating a clear correlation between high cyclin E levels and a poorer prognosis [7, 8, 10, 30]. However, our results are in agreement with a study showing that cyclin E had a prognostic value only in postmenopausal women but not in premenopausal women [31]. Nevertheless, a co-analysis of cyclin E and tumour growth pattern revealed that among the majority of patients with tumours exhibiting an exclusively infiltrative growth pattern, cyclin E did indeed confer prognostic information independent of histological grade and lymph node status.

Apart from more accurately describing the prognostic relevance of cyclin E in premenopausal breast cancer, our study emphasizes the presence of a specific subgroup of patients with relatively favourable prognosis with tumours exhibiting high histological grade, high cyclin E protein expression, negative hormone receptor status and pushing growth pattern. This observation is in agreement with findings of Putti *et al* [16], demonstrating that the presence of a pushing margin is a common morphologic feature in ER negative breast carcinomas correlating to a negative lymph node status. Many of these characteristics fit the description of the medullary-like breast tumours, a histological subtype that is more frequent in younger women and has an unexpectedly good prognosis despite the many aggressive histopathological characteristics[32]. Therefore, when assessing the prognostic value of cyclin E in premenopausal breast cancer, it is of importance to either recognize the medullary-like tumours as a separate entity, or more generally, to take the tumour growth pattern into consideration. Recently, it has been shown that there is a clear overlap between the medullary-like breast tumours, *BRCA1*-mutated tumours and tumours with a basal-like gene expression profile[33, 34]. Future studies should address how the expression of cyclin E is related to different phenotypes and clinical outcome within this entity. Interestingly, in a recent paper by Sieuwerts *et al*[11], they found the levels of cyclin E mRNA to be generally lower in medullary breast cancers compared to other subtypes. The lack of congruence between mRNA levels and the unequivocally high protein expression among the 24 medullary tumours in our material suggests that cyclin E is post-transcriptionally deregulated in these tumours.

Regarding cyclin E and tamoxifen response, our results could not confirm the previously observed association between cyclin E mRNA expression and anti-oestrogen resistance [28]. In the study by Span et al, the RFS-rates in endocrine treated ER positive patients were compared between cyclin E-low and –high groups. The small cyclin E-high group responded comparatively poorer but since no equivalent group of untreated patients was included, it is problematic to interpret the poorer response as a failure of treatment. It might be that the cyclin E-high treated patients actually did perform better compared to their untreated counterparts. Due to the lower frequency of cyclin E deregulation in ER positive

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breast cancers, it is inherently difficult to study what effect cyclin E might have on anti-oestrogen response. This, in addition to the inconclusive data presented so far, suggests that cyclin E is of minor importance as a predictor of endocrine treatment response.

In summary, we have shown that high cyclin E protein expression is strongly correlated to a pushing margin growth pattern in primary premenopausal breast cancer. Our experimental data indicating that a high proliferative activity restrains tumour cell invasiveness[15], could partially explain why many of the highly proliferative cyclin E^{high} tumours present a low infiltrative, pushing growth pattern. The present findings of a prognostic value of cyclin E in a subgroup of breast carcinomas with an exclusively infiltrative growth pattern, add important information to earlier publications on the clinical significance of cyclin E in breast cancer and might explain some of the discrepancies. However, the relationship between proliferation and invasiveness in specific tumours is most probably very complex. This issue needs to be studied in more detail in order to delineate the potential link between proliferative- and infiltrative behaviour in tumour cells. In addition, our data do not support a role for cyclin E as a predictor of tamoxifen response.

Take home messages

- Tumour growth pattern is an important property in breast cancer correlating to tumour grade and lymph node metastasis, and confers independent prognostic information.
- High cyclin E protein expression is strongly correlated to a pushing margin growth pattern in primary premenopausal breast cancer.
- Cyclin E has a prognostic value that appears to be limited to patients with breast carcinomas growing in an exclusively infiltrative manner.

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Figure legends

Figure 1

Tumour growth pattern assessment and cyclin E staining of stage II primary breast carcinomas. (A) Tumour with infiltrative growth pattern (left) and cyclin E protein expression (right). (B) Tumour with a pushing margin growth pattern (left) and cyclin E protein expression (right).

Figure 2

Associations between tumour growth patterns and clinicopathological characteristics. (A) The bars represent the fraction of tumours in the respective growth pattern groups exhibiting the indicated characteristics (total cohort). The number of patients is indicated above each bar. (B) Kaplan-Meier curve illustrating the relation between tumour growth pattern (1-4) and RFS in the untreated cohort.

Figure 3

Kaplan-Meier curves illustrating the relation between cyclin E protein levels and RFS in the untreated control cohort. (A) Total control cohort. (B) Tumours exhibiting exclusively infiltrative (1), predominantly infiltrative (2) or predominantly pushing (3) growth pattern. (C) Tumours exhibiting exclusively infiltrative or predominantly infiltrative growth pattern. (D) Tumours exhibiting exclusively infiltrative growth pattern.

Figure 4

Kaplan-Meier curve illustrating the relation between tumour growth pattern (1-4) and RFS in cyclin E^{high} untreated cohort.

Figure 5

Predicting response to adjuvant tamoxifen. (A-B) RFS for treated patients with oestrogen receptor (ER) positive- and negative tumours. (C) A Forest plot displaying hazard ratio of recurrence in patients with ER negative and -positive tumours and further, in ER positive patients according to different levels of cyclin E expression. Statistics for HR was evaluated using Cox univariate analysis.

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Table 1. Cyclin E expression in relation to patient- and tumour characteristics in the total cohort.

Variable		Cyclin E^{negative} (N=129)	Cyclin E^{low} (N=102)	Cyclin E^{high} (N=154)	p-value
Age†					0.04
	Median (range)	46 (30-57)	45 (27-55)	43 (25-57)	
Trial arm					0.05
	Tamoxifen (%)	52 (40)	53 (52)	84 (55)	
	Control (%)	77 (60)	49 (48)	70 (45)	
Tumour size					<0.01
	<20mm (%)	57 (45)	37 (36)	37 (24)	
	≥20mm (%)	71 (55)	65 (64)	117 (76)	
	n.a.	1	1		
Lymph node status					0.04
	0 (%)	25 (20)	23 (23)	55 (36)	
	1-3 (%)	76 (59)	52 (51)	68 (44)	
	≥4 (%)	27 (21)	27 (26)	30 (20)	
	n.a.	1		1	
Histological grade‡					<0.01
	1 (%)	29 (23)	12 (12)	2 (1)	
	2 (%)	73 (58)	48 (49)	32 (22)	
	3 (%)	24 (19)	38 (39)	111 (77)	
	n.a.	3	4	9	
ER status					<0.01
	Positive (%)	106 (86)	83 (84)	59 (39)	
	Negative (%)	18 (14)	16 (16)	91 (61)	
	n.a.	5	3	4	
PR status					
	Positive (%)	112 (89)	74 (82)	55 (37)	<0.01
	Negative (%)	14 (11)	16 (18)	95 (63)	
	n.a.	3	12	4	
Histological type					<0.01
	Ductal (%)	104 (82)	95 (94)	127 (84)	
	Lobular (%)	20 (16)	4 (4)	1 (1)	
	Tubular (%)	1 (1)	1 (1)		
	Medullary (%)	2 (1)		22 (14)	
	Other (%)		1 (1)	2 (1)	
	n.a.	2	1	2	
Tumour growth pattern					<0.01
	Exclusively infiltrative (%)	111 (89)	78 (81)	68 (48)	
	Predominantly infiltrative (%)	11 (9)	12 (13)	30 (21)	
	Predominantly pushing (%)	3 (2)	6 (6)	32 (22)	
	Exclusively pushing (%)			12 (9)	
	n.a.	4	6	12	

Statistics according to Chi-Square test. †Kruskal Wallis test. ‡Differentiation marker [35]. N=number of patients.

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Table 2. Tumour growth pattern in relation to patient- and tumour characteristics in the total cohort.

Variable	Exclusively infiltrative (N=349)	Predominantly infiltrative (N=62)	Predominantly pushing (N=48)	Exclusively pushing (N=13)	p-value
Age†					0.25
Median (range)	45 (26-57)	43 (31-57)	44 (31-52)	43 (35-49)	
Trial arm					0.05
Tamoxifen (%)	161 (46)	40 (65)	24 (50)	8 (62)	
Control (%)	188 (54)	22 (35)	24 (50)	5 (38)	
Tumour size					<0.01
<20mm (%)	145 (42)	17 (27)	10 (21)		
≥20mm (%)	203 (58)	45 (73)	38 (79)	13 (100)	
n.a.	1				
Lymph node status					<0.01
0 (%)	77 (22)	16 (26)	28 (58)	8 (62)	
1-3 (%)	187 (54)	30 (48)	13 (27)	3 (23)	
≥4 (%)	83 (24)	16 (26)	7 (15)	2 (15)	
n.a.	2				
Histological grade‡					<0.01
1 (%)	50 (15)	2 (3)	1 (2)		
2 (%)	191 (55)	11 (18)	2 (4)		
3 (%)	103 (30)	48 (79)	44 (94)	13 (100)	
n.a.	5				
ER status					<0.01
Positive (%)	265 (81)	29 (48)	13 (28)	1 (8)	
Negative (%)	63 (19)	31 (52)	34 (72)	11 (92)	
n.a.					
PR status					<0.01
Positive (%)	259 (83)	25 (42)	12 (26)	1 (8)	
Negative (%)	55 (17)	34 (58)	34 (74)	11 (92)	
n.a.					
Histological type					<0.01
Ductal (%)	298 (86)	62 (100)	31 (66)	3 (23)	
Lobular (%)	42 (12)		1 (2)		
Tubular (%)	5 (1)				
Medullary (%)			15 (32)	10 (77)	
Other (%)	2 (1)				
n.a.	5		1		

Statistics according to Chi-Square test. †Mann-Whitney-U test. ‡Differentiation marker [35]. N=number of patients.

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Table 3. Hazard ratios for RFS in patients in the control cohort calculated with Cox uni- and multivariate analysis (N=288). All parameters identified as significant by univariate analysis were included in the multivariate analysis.

	Univariate			Multivariate		
Variable†	HR	95% CI	p-value	HR	95% CI	p-value
Grade‡	1.63	1.25-2.13	<0.01	1.77	1.22-2.59	<0.01
Node status	1.88	1.48-2.38	<0.01	1.74	1.28-2.38	<0.01
Tumour size	1.24	0.89-1.73	0.21			
Growth pattern	0.73	0.55-0.97	0.03	0.56	0.39-0.80	<0.01
Cyclin E	1.09	0.87-1.36	0.47			

† Variables were grouped as shown in Table 1 and 2.
‡ Differentiation marker [35].

Table 4. Hazard ratios for RFS in patients in the control cohort with tumours showing an exclusively infiltrative growth pattern (N=188), calculated with Cox uni- and multivariate. All parameters identified as significant by univariate analysis were included in the multivariate analysis.

	Univariate			Multivariate		
Variable†	HR	95% CI	p-value	HR	95% CI	p-value
Grade‡	2.45	1.77-3.39	<0.01	1.64	1.10-2.42	0.01
Node status	1.78	1.30-2.42	<0.01	2.10	1.44-3.05	<0.01
Tumour size	1.54	1.04-2.27	0.03	1.26	0.80-1.98	0.31
Cyclin E	1.45	1.12-1.89	0.01	1.40	1.05-1.87	0.02

† Variables were grouped as shown in Table 1 and 2.
‡ Differentiation marker [35].

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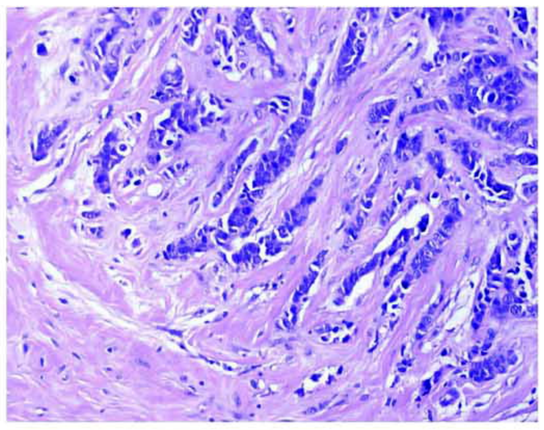
Figure 1.

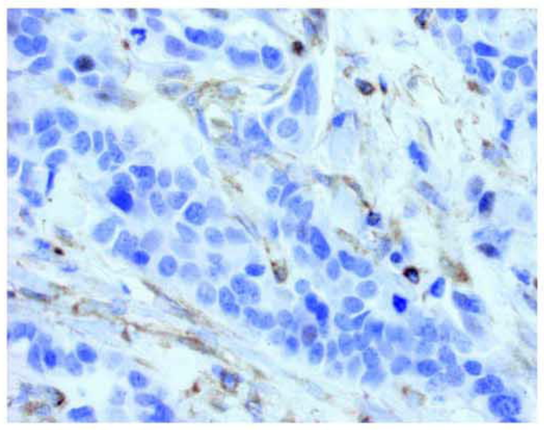
A

PT-2019

Infiltrative growth pattern



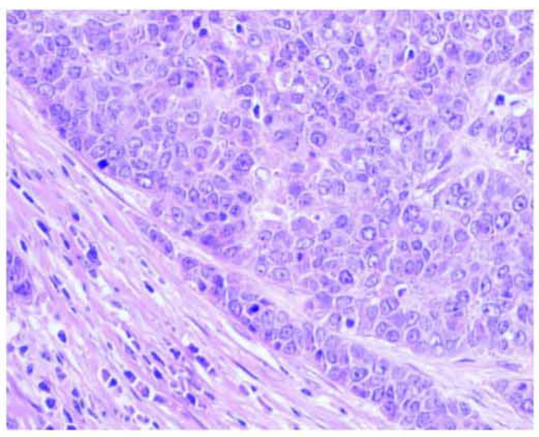
Cyclin E



B

PT-2305

Pushing margin growth pattern



Cyclin E

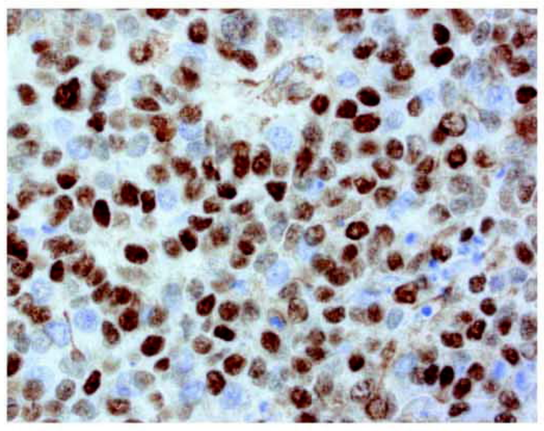


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Figure 2

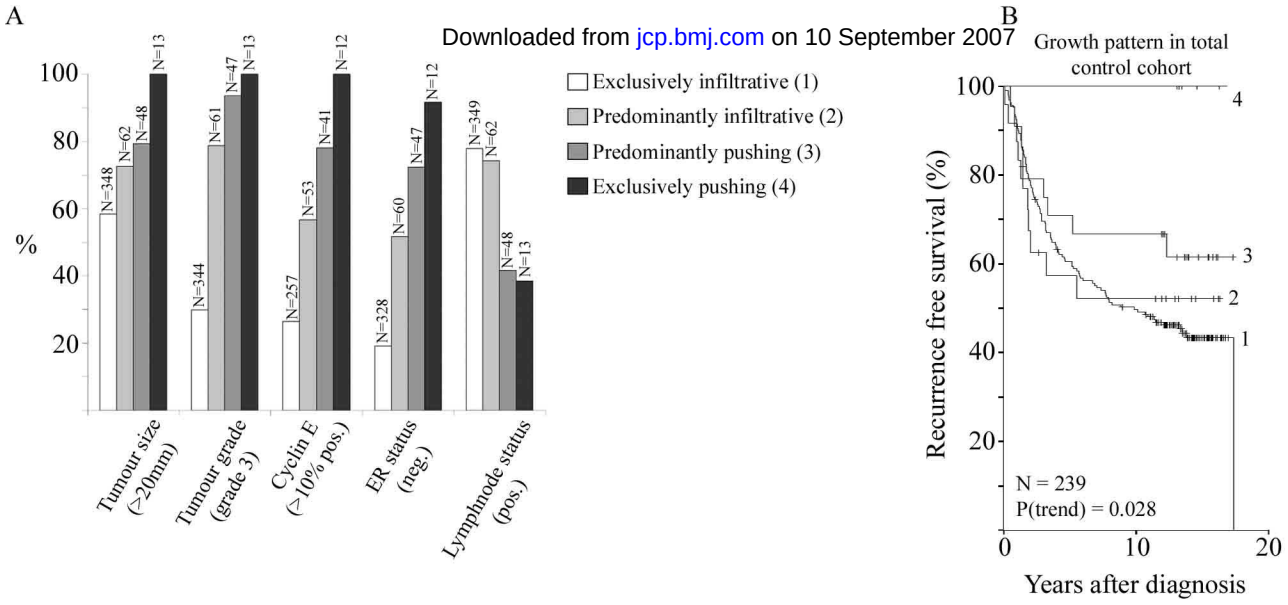


Figure 3

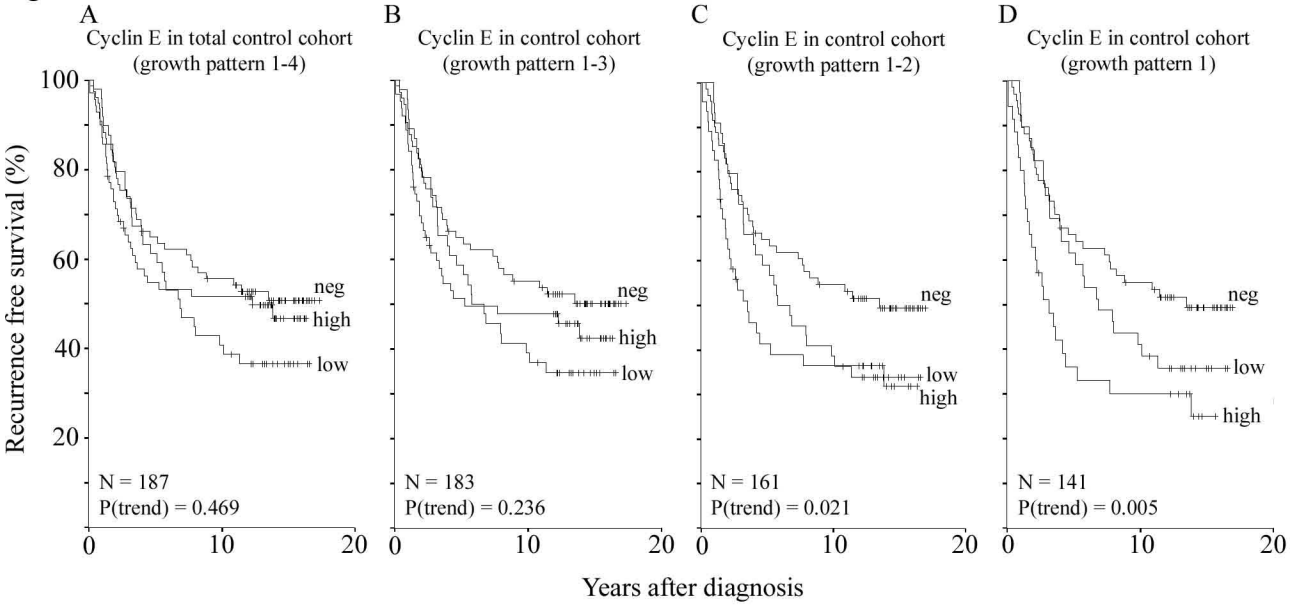
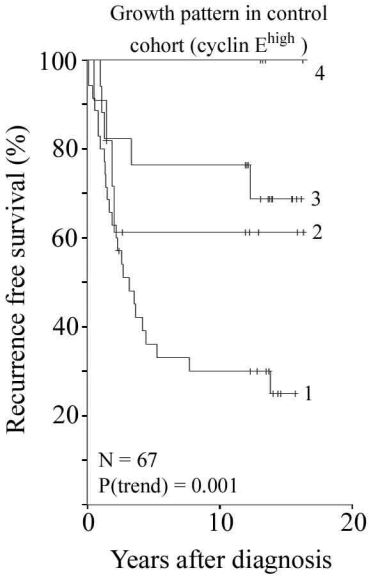


Figure 4



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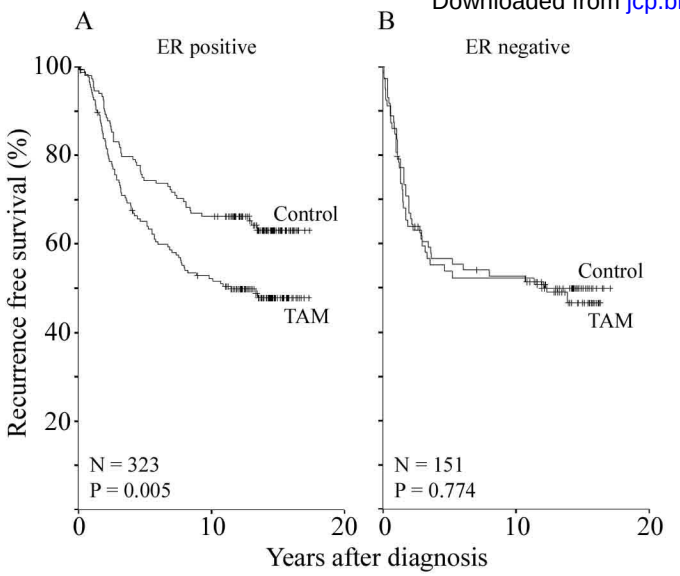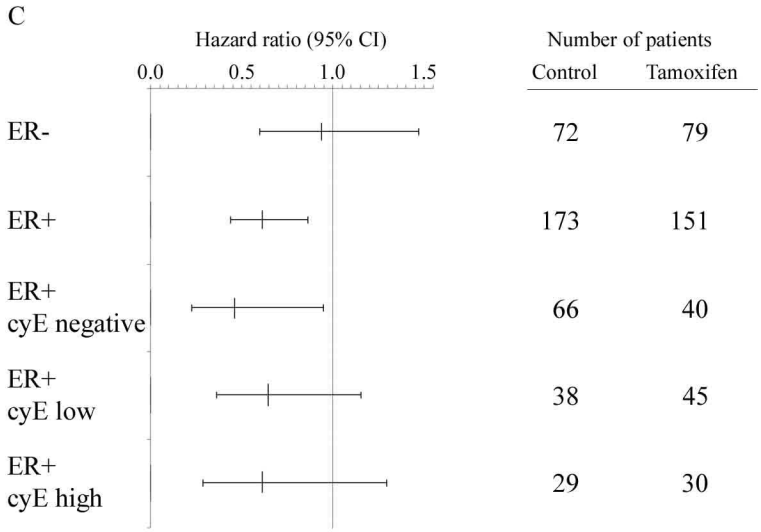
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