LUND UNIVERSITY

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

Berglund, Pontus; Stighall, Maria; Jirström, Karin; Rydén, Lisa; Fernö, Mårten; Nordenskjold, Bo; Landberg, Göran

Published in:
Journal of Clinical Pathology

DOI:
10.1136/jcp.2007.047688

2008

Link to publication

Citation for published version (APA):
Berglund, P., Stighall, M., Jirström, K., Rydén, L., Fernö, M., Nordenskjold, B., & Landberg, G. (2008). Cyclin E confers a prognostic value in premenopausal breast cancer patients with tumours exhibiting an infiltrative growth pattern. *Journal of Clinical Pathology*, *61*, 184-191. https://doi.org/10.1136/jcp.2007.047688

Total number of authors:
7

General rights
Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.
• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: https://creativecommons.org/licenses/

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Download date: 12. Oct. 2026

Downloaded from jcp.bmj.com on 10 September 2007

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

Pontus Berglund, Maria Stighall, Karin Jirström, Lisa Rydén, Mårten Fernö, Bo Nordenskjöld and Göran Landberg

J. Clin. Pathol. published online 4 May 2007;
doi:10.1136/jcp.2007.047688

Updated information and services can be found at:
http://jcp.bmj.com/cgi/content/abstract/jcp.2007.047688v1

These include:

Rapid responses You can respond to this article at:
http://jcp.bmj.com/cgi/eletter-submit/jcp.2007.047688v1

Email alerting service Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article

Notes

Online First contains unedited articles in manuscript form that have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Online First articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Online First articles must include the digital object identifier (DOIs) and date of initial publication.

To order reprints of this article go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to *Journal of Clinical Pathology* go to:
http://journals.bmj.com/subscriptions/

Downloaded from jcp.bmj.com on 10 September 2007
JCP Online First, published on May 4, 2007 as 10.1136/jcp.2007.047688

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

Pontus Berglund[1†], Maria Stighall[1†], Karin Jirström[1], Lisa Rydén[2], Mårten Fernö[3], Bo Nordenskjöld[4], and Göran Landberg[1‡]

[1]Center for Molecular Pathology, Department of Laboratory Medicine, Lund University, Malmö University Hospital, Malmö, Sweden.
[2]Department of Surgery, Helsingborg Hospital, Helsingborg, Sweden.
[3]Department of Oncology, Lund University Hospital, Lund, Sweden.
[4]Department of Oncology, Linköping University Hospital, Linköping, Sweden.
[†]Both authors contributed equally to the work.

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence (or non exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd to permit this article (if accepted) to be published in JCP and any other BMJPGL products and sublicences such use and exploit all subsidiary rights, as set out in our licence (http://JCP.bmjjournals.com/ifora/licence.pdf).

[‡]Correspondence: Göran Landberg, Center for Molecular Pathology, Department of Laboratory Medicine, Lund University, Malmö University Hospital, SE-205 02 Malmö, Sweden
Fax: +46 40 337 063
Phone: +46 40 331 953
Email: goran.landberg@med.lu.se

Copyright Article author (or their employer) 2007. Produced by BMJ Publishing Group Ltd under licence.

Downloaded from jcp.bmj.com on 10 September 2007

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

Downloaded from jcp.bmj.com on 10 September 2007

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

Downloaded from jcp.bmj.com on 10 September 2007

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.

Downloaded from jcp.bmj.com on 10 September 2007

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

Downloaded from jcp.bmj.com on 10 September 2007

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).

Downloaded from jcp.bmj.com on 10 September 2007

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

Downloaded from jcp.bmj.com on 10 September 2007

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.

Downloaded from jcp.bmj.com on 10 September 2007

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

Downloaded from jcp.bmj.com on 10 September 2007

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.

Acknowledgements

This study was supported by grants from the Swedish Research Council, the Swedish Cancer Society, Gunnar, Arvid and Elisabeth Nilsson Cancer Foundation, Lund University Research Funds, Per-Eric and Ulla Schybergs Foundation, Malmö University Hospital Research and Cancer Funds and Swegene/Wallenberg Consortium North. We are grateful for the work of the South- and Southeast Swedish Breast Cancer groups that has enabled us to use the breast cancer material. The authors would like to thank Elise Nilsson for skilful technical assistance in performing immunohistochemistry.

Downloaded from jcp.bmj.com on 10 September 2007

References

1. Ohtsubo M, Theodoras A, Schumacher J, Roberts J, Pagano M: Human cyclin E, a nuclear protein essential for the G1-to-S phase transition, Mol. Cell. Biol. 1995, 15:2612-2624
2. Resnitzky D, Reed S: Different roles for cyclins D1 and E in regulation of the G1-to-S transition, Mol. Cell. Biol. 1995, 15:3463-3469
3. Spruck CH, Won KA, Reed SI: Deregulated cyclin E induces chromosome instability, Nature 1999, 401:297-300
4. Ekholm-Reed S, Mendez J, Tedesco D, Zetterberg A, Stillman B, Reed SI: Deregulation of cyclin E in human cells interferes with prereplication complex assembly, J. Cell Biol. 2004, 165:789-800
5. Kawamura K, Izumi H, Ma Z, Ikeda R, Moriyama M, Tanaka T, Nojima T, Levin LS, Fujikawa-Yamamoto K, Suzuki K, Fukasawa K: Induction of Centrosome Amplification and Chromosome Instability in Human Bladder Cancer Cells by p53 Mutation and Cyclin E Overexpression, Cancer Res 2004, 64:4800-4809
6. Bortner DM, Rosenberg MP: Induction of mammary gland hyperplasia and carcinomas in transgenic mice expressing human cyclin E, Mol Cell Biol 1997, 17:453-459
7. Keyomarsi K, O'Leary N, Molnar G, Lees E, Fingert HJ, Pardee AB: Cyclin E, a potential prognostic marker for breast cancer, Cancer Res 1994, 54:380-385
8. Nielsen NH, Arnerlov C, Emdin SO, Landberg G: Cyclin E overexpression, a negative prognostic factor in breast cancer with strong correlation to oestrogen receptor status, Br J Cancer 1996, 74:874-880
9. Nielsen NH, Arnerlov C, Cajander S, Landberg G: Cyclin E expression and proliferation in breast cancer, Anal Cell Pathol 1998, 17:177-188
10. Porter PL, Malone KE, Heagerty PJ, Alexander GM, Gatti LA, Firpo EJ, Daling JR, Roberts JM: Expression of cell-cycle regulators p27Kip1 and cyclin E, alone and in combination, correlate with survival in young breast cancer patients, Nat Med 1997, 3:222-225
11. Sieuwerts AM, Look MP, Meijer-van Gelder ME, Timmermans M, Trapman AM, Garcia RR, Arnold M, Goedheer AJ, de Weerd V, Portengen H, Klijn JG, Foekens JA: Which cyclin E prevails as prognostic marker for breast cancer? Results from a retrospective study involving 635 lymph node-negative breast cancer patients, Clin Cancer Res 2006, 12:3319-3328

Downloaded from jcp.bmj.com on 10 September 2007

12. Donnellan R, Kleinschmidt I, Chetty R: Cyclin E immunoexpression in breast ductal carcinoma: pathologic correlations and prognostic implications, Hum Pathol 2001, 32:89-94
13. Kuhling H, Alm P, Olsson H, Ferno M, Baldetorp B, Parwaresch R, Rudolph P: Expression of cyclins E, A, and B, and prognosis in lymph node-negative breast cancer, J Pathol 2003, 199:424-431
14. Porter PL, Barlow WE, Yeh IT, Lin MG, Yuan XP, Donato E, Sledge GW, Shapiro CL, Ingle JN, Haskell CM, Albain KS, Roberts JM, Livingston RB, Hayes DF: p27(Kip1) and cyclin E expression and breast cancer survival after treatment with adjuvant chemotherapy, J Natl Cancer Inst 2006, 98:1723-1731
15. Berglund P, Stighall M, Jirstrom K, Borgquist S, Sjolander A, Hedenfalk I, Landberg G: Cyclin E overexpression obstructs infiltrative behavior in breast cancer: a novel role reflected in the growth pattern of medullary breast cancers, Cancer Res 2005, 65:9727-9734
16. Putti TC, El-Rehim DM, Rakha EA, Paish CE, Lee AH, Pinder SE, Ellis IO: Estrogen receptor-negative breast carcinomas: a review of morphology and immunophenotypical analysis, Mod Pathol 2004,
17. Pedersen L: Medullary carcinoma of the breast, APMIS Suppl 1997, 75:1-31
18. EBCTGC: Systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy. 133 randomised trials involving 31,000 recurrences and 24,000 deaths among 75,000 women. Early Breast Cancer Trialists' Collaborative Group, Lancet 1992, 339:71-85
19. EBCTCG: Tamoxifen for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group, Lancet 1998, 351:1451-1467
20. Ali S, Coombes RC: Endocrine-responsive breast cancer and strategies for combating resistance, Nat Rev Cancer 2002, 2:101-112
21. Zwijsen RM, Wientjens E, Klompmaker R, van der Sman J, Bernards R, Michalides RJ: CDK-independent activation of estrogen receptor by cyclin D1, Cell 1997, 88:405-415
22. McMahon C, Suthiphongchai T, DiRenzo J, Ewen ME: P/CAF associates with cyclin D1 and potentiates its activation of the estrogen receptor, Proc Natl Acad Sci U S A 1999, 96:5382-5387
23. Lamb J, Ladha MH, McMahon C, Sutherland RL, Ewen ME: Regulation of the functional interaction between cyclin D1 and the estrogen receptor, Mol Cell Biol 2000, 20:8667-8675
24. Kurokawa H, Arteaga CL: ErbB (HER) receptors can abrogate antiestrogen action in human breast cancer by multiple signaling mechanisms, Clin Cancer Res 2003, 9:511S-515S
25. Dhillon NK, Mudryj M: Ectopic expression of cyclin E in estrogen responsive cells abrogates antiestrogen mediated growth arrest, Oncogene 2002, 21:4626-4634
26. Akli S, Zheng PJ, Multani AS, Wingate HF, Pathak S, Zhang N, Tucker SL, Chang S, Keyomarsi K: Tumor-specific low molecular weight forms of cyclin E induce genomic instability and resistance to p21, p27, and antiestrogens in breast cancer, Cancer Res 2004, 64:3198-3208
27. Hui R, Finney GL, Carroll JS, Lee CS, Musgrove EA, Sutherland RL: Constitutive overexpression of cyclin D1 but not cyclin E confers acute resistance to antiestrogens in T-47D breast cancer cells, Cancer Res 2002, 62:6916-6923
28. Span PN, Tjan-Heijnen VC, Manders P, Beex LV, Sweep CG: Cyclin-E is a strong predictor of endocrine therapy failure in human breast cancer, Oncogene 2003, 22:4898-4904
29. Ryden L, Jonsson PE, Chebil G, Dufmats M, Ferno M, Jirstrom K, Kallstrom AC, Landberg G, Stal O, Thorstenson S, Nordenskjold B: Two years of adjuvant tamoxifen in premenopausal patients with breast cancer: a randomised, controlled trial with long-term follow-up, Eur J Cancer 2005, 41:256-264

Downloaded from jcp.bmj.com on 10 September 2007

30. Keyomarsi K, Tucker SL, Buchholz TA, Callister M, Ding Y, Hortobagyi GN, Bedrosian I, Knickerbocker C, Toyofuku W, Lowe M, Herliczek TW, Bacus SS: Cyclin E and survival in patients with breast cancer, N Engl J Med 2002, 347:1566-1575
31. Rudolph P, Kuhling H, Alm P, Ferno M, Baldetorp B, Olsson H, Parwaresch R: Differential prognostic impact of the cyclins E and B in premenopausal and postmenopausal women with lymph node-negative breast cancer, Int J Cancer 2003, 105:674-680
32. Ridolfi RL, Rosen PP, Port A, Kinne D, Mike V: Medullary carcinoma of the breast: a clinicopathologic study with 10 year follow-up, Cancer 1977, 40:1365-1385
33. Jacquemier J, Padovani L, Rabayrol L, Lakhani SR, Penault-Llorca F, Denoux Y, Fiche M, Figueiro P, Maisongrosse V, Ledoussal V, Martinez Penuela J, Udvarhely N, El Makdissi G, Ginestier C, Geneix J, Charafe-Jauffret E, Xerri L, Eisinger F, Birnbaum D, Sobol H: Typical medullary breast carcinomas have a basal/myoepithelial phenotype, J Pathol 2005, 207:260-268
34. Bertucci F, Finetti P, Cervera N, Charafe-Jauffret E, Mamessier E, Adelaide J, Debono S, Houvenaeghel G, Maraninchi D, Viens P, Charpin C, Jacquemier J, Birnbaum D: Gene expression profiling shows medullary breast cancer is a subgroup of basal breast cancers, Cancer Res 2006, 66:4636-4644
35. Elston CW, Ellis IO: Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up, Histopathology 1991, 19:403-410

Downloaded from jcp.bmj.com on 10 September 2007

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.

Downloaded from jcp.bmj.com on 10 September 2007

Downloaded from jcp.bmj.com on 10 September 2007

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.

Downloaded from jcp.bmj.com on 10 September 2007

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.

Downloaded from jcp.bmj.com on 10 September 2007

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].

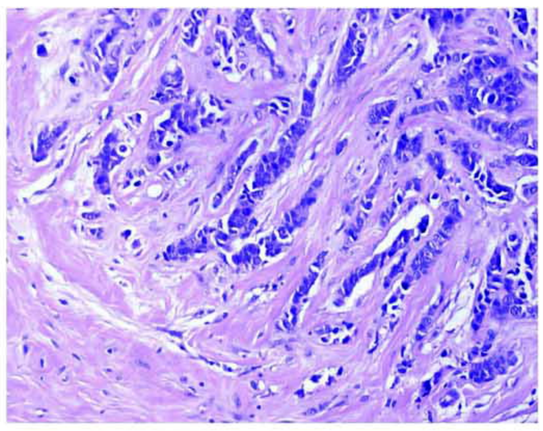
Downloaded from jcp.bmj.com on 10 September 2007

Figure 1.

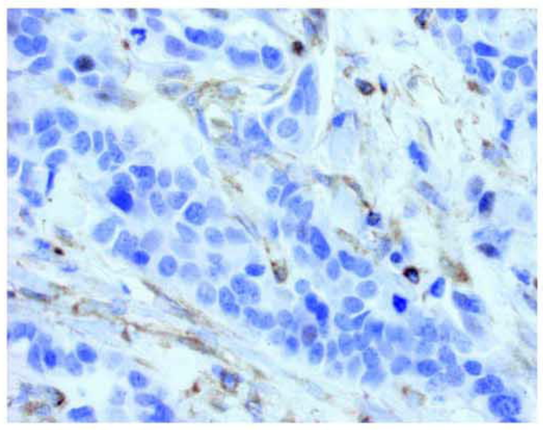
A

PT-2019

Infiltrative growth pattern



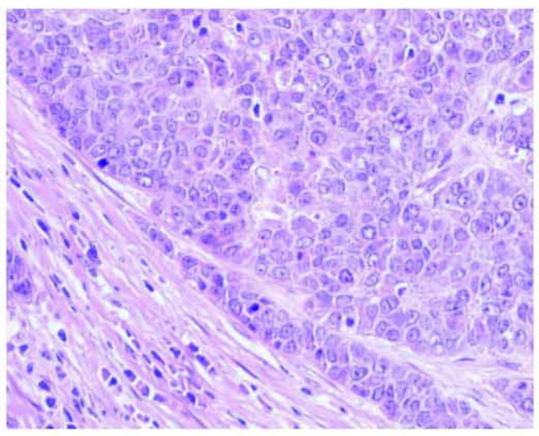
Cyclin E



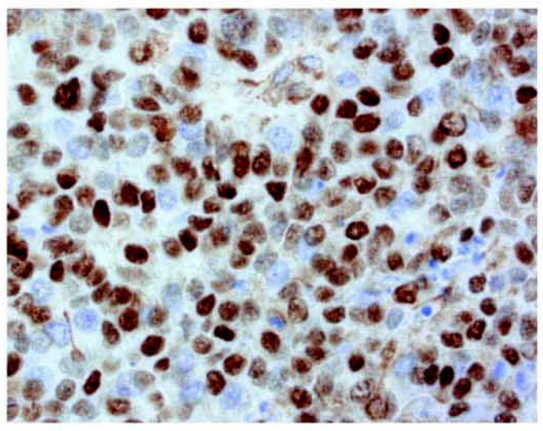
B

PT-2305

Pushing margin growth pattern



Cyclin E



Downloaded from jcp.bmj.com on 10 September 2007

Figure 2

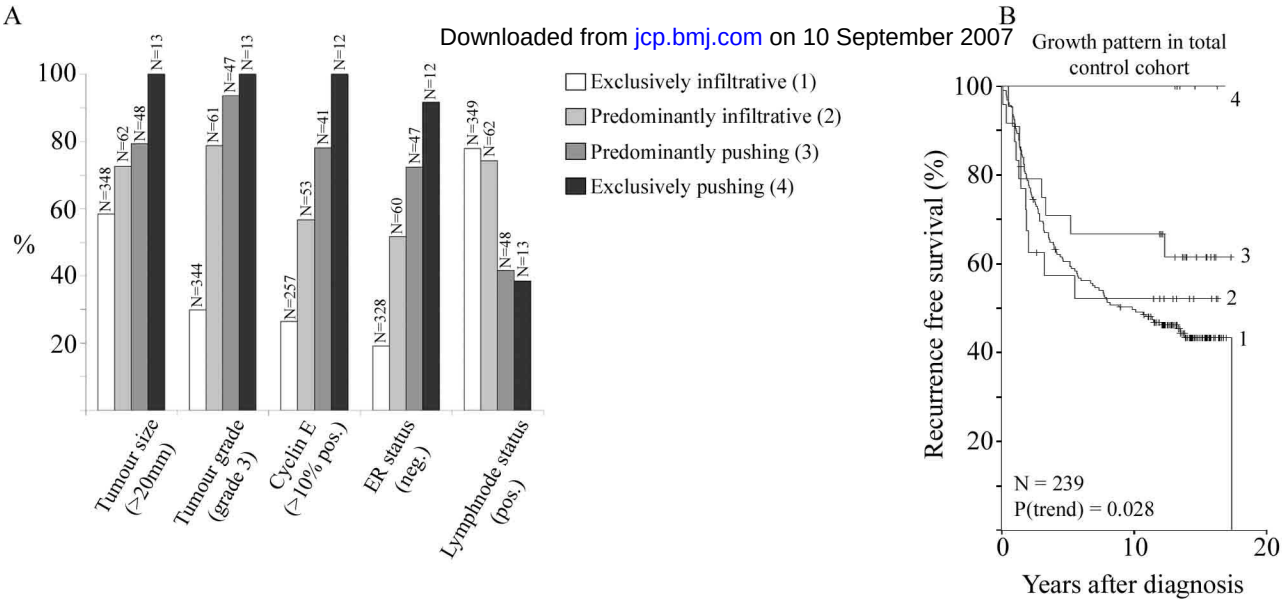


Figure 3

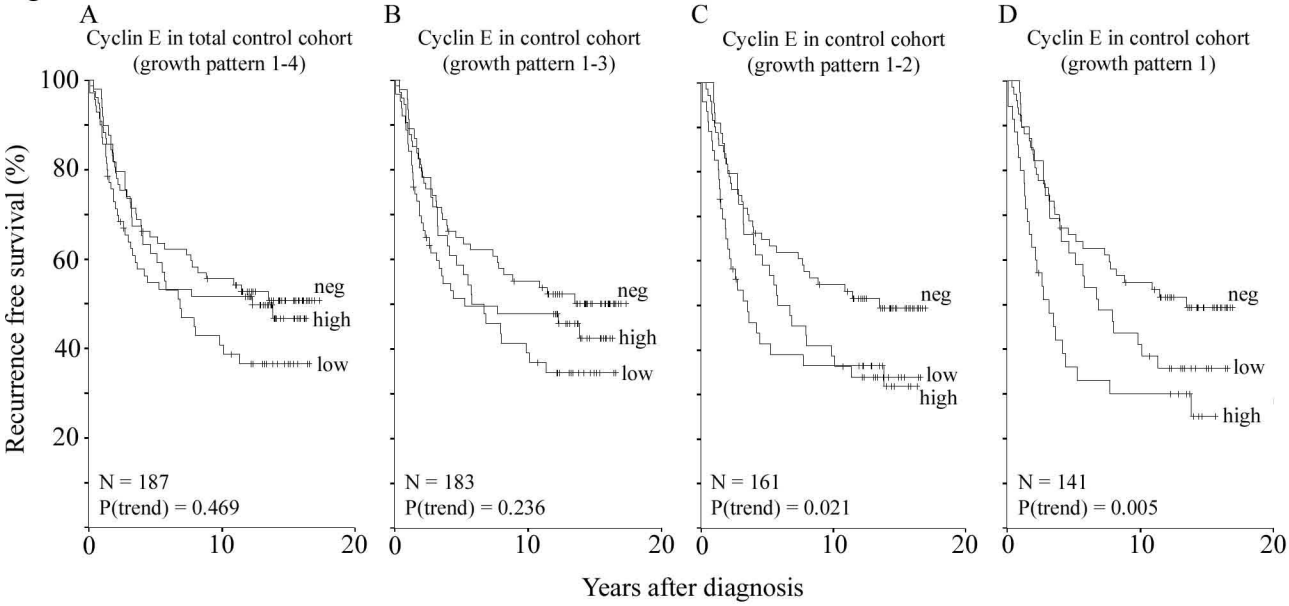
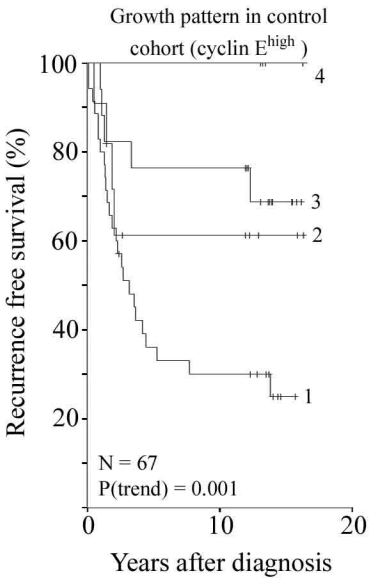


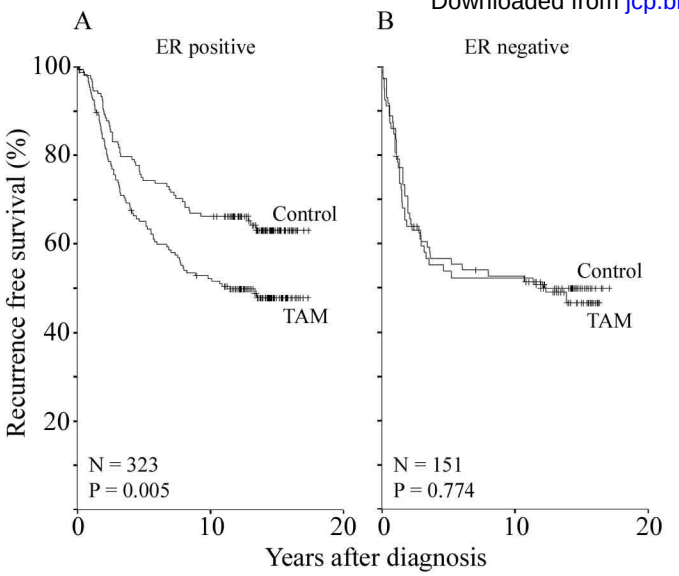
Figure 4



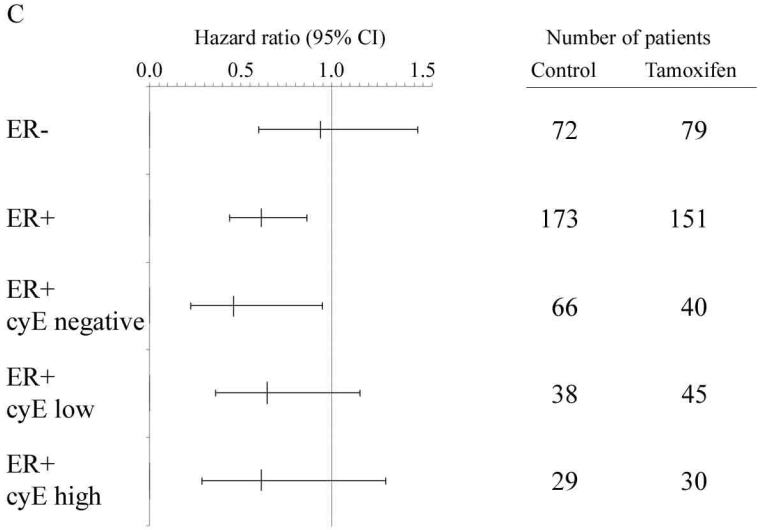
Downloaded from jcp.bmj.com on 10 September 2007

Figure 5

Downloaded from jcp.bmj.com on 10 September 2007



C



	Number of patients	
	Control	Tamoxifen
ER-	72	79
ER+	173	151
ER+ cyE negative	66	40
ER+ cyE low	38	45
ER+ cyE high	29	30