

CD24 Expression Is a New Prognostic Marker in Breast Cancer

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ABSTRACT

Purpose: *CD24* is expressed in hematological malignancies as well as in a large variety of solid tumors including breast cancer. We aimed to evaluate *CD24* protein expression in breast cancer and to correlate to clinicopathological data including patient survival.

Experimental Design: Primary breast carcinomas (201) with a mean clinical follow-up time of 53 months were immunostained using a monoclonal *CD24* antibody (Ab-2, clone 24C02). The staining was evaluated as negative versus positive for statistical analysis.

Results: In invasive breast carcinomas, *CD24* expression was observed in 84.6% of cases. In univariate survival analyses, a significant association of *CD24* expression with shortened patient overall survival (5-year survival rate 91.9% versus 83.8%; $P = 0.031$; log rank test) and disease-free survival (5-year progression rate 88.3% versus 57.0%; $P = 0.0008$) was demonstrated. In multivariate analyses *CD24*, tumor grading and nodal status were significant prognostic parameters for shortened disease-free survival.

Conclusions: Our data suggest that *CD24* expression in primary breast cancer as detected by immunohistochemistry might be a new marker for a more aggressive breast cancer biology.

INTRODUCTION

Breast cancer is the most common malignant tumor of females in the western world. A rising incidence of this disease was observed in Germany in the last 20 years, and 46,000 new cases were diagnosed in 1998. In the United States alone, 203,500 new cases were expected for 2002 (1, 2). The incidence

of breast cancer remains high, and the clinical courses are highly variable. It is of general importance to predict the biology of the tumor and, thus, the course of the disease in the individual patient to ensure adequate therapy and patient surveillance. Conventional prognostic and predictive markers for breast cancer are nodal status, tumor grade, tumor size, and tumor type (3, 4). Additionally, molecular markers are being sought and established to allow for a refined classification of prognosis, especially in patient subgroups whose outcome can only insufficiently be predicted by conventional parameters. Among others, candidate genes of current interest are *telomerase*, *kallikrein 5*, *urokinase plasminogen activator* and its inhibitor (*PAI-1*), *tissue inhibitor of metalloproteinases 1*, *Ep-Cam*, *c-erbB2*, and *osteopontin* (5–10). A novel prognostic marker gene we identified recently in epithelial ovarian cancer, non-small cell lung cancer, and prostate cancer is *CD24* (11–13).

CD24 is a small, heavily glycosylated mucin-like glycoliposphatidyl-inositol-linked cell surface protein that is expressed in a wide variety of human malignancies, e.g., B cell lymphoma, renal cell carcinoma, small cell and non-small cell lung carcinoma, nasopharyngeal carcinoma, hepatocellular carcinoma, bladder carcinoma, glioma, epithelial ovarian cancer, and breast cancer (12–34). Functionally, *CD24* expression might enhance the metastatic potential of tumor cells, because *CD24* has been identified as an alternative ligand of *P-selectin*, an adhesion receptor on activated endothelial cells and platelets (35–39). Fogel *et al.* (31) described *CD24* expression in breast cancer in a frozen section-based pilot study with 29 cases that did not allow for a detailed analysis. We aimed to investigate the expression of *CD24* in a larger tumor collection of breast cancer and to evaluate its prognostic significance.

MATERIALS AND METHODS

Cell Lines and Cytofluorography. The A125 adenocarcinoma cell line and *CD24*-transfected A125 cells have been described recently (37). The monoclonal *CD24* antibody (Ab-2, clone 24C02/SN3b) was purchased from Neomarkers (Fremont, CA). Staining of the cells with this antibody and phycoerythrin-conjugated goat antibodies to mouse immunoglobulins (SERVA, Heidelberg, Germany) has been performed as described (39) and was analyzed with a FACScan (Becton Dickinson, Heidelberg, Germany).

Patients. Our study included 201 patients with primary breast cancer who were diagnosed at the Institute of Pathology, Charité University Hospital, between 1991 and 1997. Patient age at the time of diagnosis ranged from 30 to 87 with a median of 59 years. Follow-up data including overall survival, and disease recurrence or progression times were available for all of the cases. The average observation time for overall survival was 65 months for patients still alive at the time of analysis, and ranged from 5 to 129 months. Thirty-four patients (16.9%) died during follow-up, and 68 patients (33.8%) experienced disease progression defined by either metastatic or local recurrent disease. Adjuvant therapy was administered as follows: 20 patients

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did not receive any adjuvant therapy, 29 patients received radiotherapy only, 26 patients had CTX² only, 10 patients had radio- and CTX, 49 patients were treated with TAM exclusively, 16 patients had TAM after CTX, 36 patients had TAM after radiotherapy, and 3 patients had a combination of radio- and CTX plus TAM. For 12 patients no data on adjuvant therapy was accessible. For statistical analysis of the impact of therapy, we arranged the patients into two groups. The first group received either no or local therapy/radiotherapy (49 cases), or systemic therapy excluding TAM (36 cases). The second group had received TAM with or without an additional systemic or local therapy (104 cases). The selection of cases for this study was based on availability of tissue, and these were not stratified for any known preoperative or pathological prognostic factors. Cases with systemic disease (M1) at the time of diagnosis were excluded. Tumor histology was determined according to the criteria of the WHO. The stage of tumors was assessed according to Unio Internationale Contra Cancrum (40). Tumors were graded according to Bloom and Richardson in the modification of Elston and Ellis (41). Data regarding the estrogen receptor status and the expression of *c-erbB2* were taken from the archival pathology reports. Estrogen receptor positivity was defined as an immune reactive score >3. Overexpression of *c-erbB2* was defined according to the clinical trial assay (2+, 3+) as recommended in the hercep-test (DAKO). The clinicopathological characteristics of the tumor collection are described in Table 1.

Immunohistochemistry. Formalin-fixed, paraffin embedded tissue was freshly cut (4 μm). The sections were mounted on superfrost slides (Menzel Gläser, Braunschweig, Germany), dewaxed with xylene, and gradually hydrated. Antigen retrieval was achieved by pressure cooking in 0.01 M citrate buffer for 5 min. The primary CD24-antibody (Ab-2, clone 24C02; Neomarkers) was diluted 1:100 using a background reducing dilution buffer (DAKO, Hamburg, Germany). No other blocking agents were used. The primary antibody was incubated at room temperature for 2 h. As a negative control, four slides were processed without primary antibody. Detection took place by the conventional labeled-streptavidin-biotin (DAKO) method with alkaline phosphatase as the reporting enzyme according to the manufacturer's instructions. Fast-Red (Sigma-Aldrich, Munich, Germany) served as chromogen. Afterward, the slides were briefly counterstained with hematoxylin and aqueously mounted.

Evaluation of the Immunohistochemical Stainings. The immunostainings were examined independently by a doctoral candidate (E. M.) and two clinical pathologists (G. K., H. G.), who were blinded to patient outcome. We aimed to keep our scoring system of the *CD24* staining as simple as possible to minimize interobserver variability and to enhance the reproducibility of our findings in future studies. We evaluated the membranous and the cytoplasmic staining intensity of *CD24* separately. Negative cases had to show definitely no *CD24* immunoreactivity in any part of the tumor. All of the other cases, beginning with a weak but unequivocal staining of tumor cells, were defined as positive. The preliminary analysis of

Table 1 Clinicopathological parameters of the tumor set

		Number of cases	%
Histology	Total number	201	100
	Invasive ductal carcinomas	163	81.1
	Invasive lobular carcinomas	21	10.4
	Mixed (ILC and IDC)	1	0.5
	Invasive carcinomas		
	Mucinous	8	4
	Tubular	3	1.5
	Papillary	1	0.5
	Medullary	2	1
	Adenoid-cystic	2	1
pT stage	pT1	127	63.2
	pT2	58	28.8
	pT3	7	3.5
	pT4	9	4.5
Nodal status	pNx	3	1.5
	pN0	94	46.8
	pN1	86	42.8
	pN2	18	8.9
Stage (UICC)	I	96	47.8
	II	74	36.8
	III	31	15.4
Tumor grade	G1	47	23.4
	G2	104	51.7
	G3	50	24.9
Age	<60	105	52.2
	≥60	96	47.8
Adjuvant therapy (n = 189)	None/radiotherapy	49	24.4
	Chemotherapy only	36	17.9
Estrogen receptor (n = 181)	Tamoxifen/± chemotherapy	104	51.7
	Negative	57	31.5
c-erbB2 (n = 168)	Positive	124	68.5
	0, 1+	121	72
	2+, 3+	47	28

membranous *CD24* staining did not reveal any significant associations with prognostic or other tumor parameters. Therefore, we concentrated on the qualities of total *CD24* and cytoplasmic *CD24* for statistical analysis. All of the cases with different scorings were discussed at a multiheaded microscope until consensus was reached.

Statistical Analysis. The data were compiled with the software package SPSS, version 10.0. Fisher's exact and χ^2 tests were used to assess the statistical significance of the correlation between expression of *CD24* and clinicopathological parameters. Univariate survival analysis was performed according to Kaplan-Meier, and differences in survival curves were assessed with the log rank test. Multivariate survival analysis was performed on all of the parameters that were found to be significant on univariate analysis using the Cox regression model. *P*s <0.05 were considered significant. All of the statistics were accredited by the head biostatistician of the Tumor Centre, Charité University Hospital (J. B.).

RESULTS

Evaluation of Antibody Specificity

We examined the reactivity of two monoclonal antibodies to *CD24* (ML5 and Ab-2) using *CD24*-transfected cell lines. As

² The abbreviations used are: CTX, chemotherapy; TAM, tamoxifen.

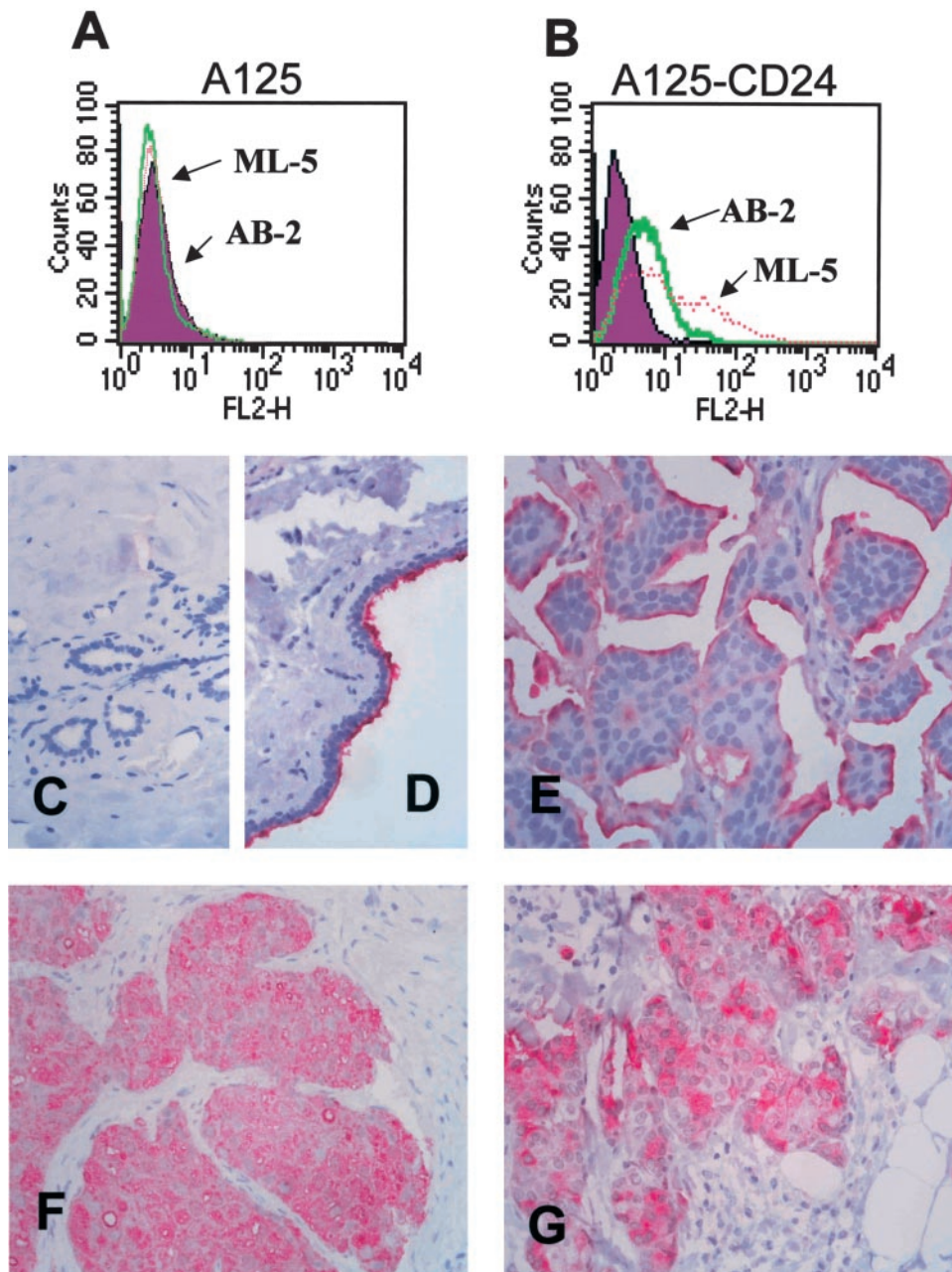


Fig. 1 CD24 immunohistochemistry of breast tissue and breast cancer. *A*, fluorescence-activated cell sorter analysis of the CD24-negative cell line A125 using the antibodies ML-5 and Ab-2. *B*, matching fluorescence-activated cell sorter analysis of the CD24-transfected cell line CD24-A125. *C*, a terminal duct without CD24 staining. *D*, dilated ducts of benign mastopathy with a strong apical staining. *E*, invasive (papillary) carcinoma with CD24 expression. *F* and *G*, strong cytoplasmic CD24 expression in invasive carcinoma, with a mosaic pattern in *G*.

shown in Fig. 1, both antibodies demonstrated a stronger staining in the CD24-transfected cell line (Fig. 1B), whereas the nontransfected cell line showed no significant signal (Fig. 1A).

CD24 Immunostaining in Breast Tissue

In normal breast tissue, CD24 was rarely expressed, only focally an apically accentuated membranous staining of ductal epithelium was noted (Fig. 1C). Dilated ducts virtually always displayed a strong apical immunoreactivity (Fig. 1D). This apical staining pattern was also observed in a fraction of carcinomas. In intraductal and invasive carcinomas an additional cytoplasmic staining quality was observed, which was often

membranously accentuated at the circumference of the cell, or showed a mosaic pattern (Fig. 1, E–G). In invasive carcinomas, we observed membranous CD24 expression in 55.7% and a cytoplasmic CD24 expression in 76.1%. Both staining qualities showed a rate of concordance of 62.7%. In total, 84.6% of tumors expressed CD24.

We did not find any significant association of overall CD24 staining intensity with patient age, tumor type, tumor size (pT), tumor grading, estrogen receptor, or c-erbB2 expression. Only a positive nodal status ($P = 0.018$) was linked to CD24 positivity (Table 2). Comparable associations were found for cytoplasmic CD24 staining. No correlations between membranous CD24

Table 2 Relationship between total *CD24* expression and various clinicopathological factors

Characteristic	All cases	<i>CD24</i> negative	<i>CD24</i> positive	<i>P</i>
All carcinomas	201 (100%)	31 (15.4%)	170 (84.6%)	
Age at surgery (yr.)				0.698
≤60	105 (100%)	15 (14.3%)	90 (85.7%)	
>60	96 (100%)	16 (16.7%)	80 (83.3%)	
Histological type				0.321
Ductal carcinoma	163 (100%)	26 (16.0%)	137 (84.0%)	
Lobular carcinoma	21 (100%)	1 (4.8%)	20 (95.2%)	
pT				0.103 ^a
pT1	127 (100%)	24 (18.9%)	103 (81.1%)	
pT2	58 (100%)	4 (6.9%)	54 (93.1%)	
pT3/4	16 (100%)	3 (18.7%)	13 (81.3%)	
pN				0.018
pN0	94 (100%)	21 (22.3%)	73 (77.7%)	
pN1+	104 (100%)	10 (9.6%)	94 (90.4%)	
UICC stage				0.053 ^a
I	96 (100%)	21 (21.9%)	75 (78.1%)	
II	74 (100%)	7 (9.5%)	67 (90.5%)	
III	31 (100%)	3 (9.7%)	28 (90.3%)	
Histological grade				0.445 ^a
G1	47 (100%)	10 (21.3%)	37 (78.7%)	
G2	104 (100%)	14 (13.5%)	90 (86.5%)	
G3	50 (100%)	7 (14%)	43 (86%)	
Estrogen receptor				0.369
Negative	57 (100%)	6 (10.5%)	51 (89.5%)	
Positive	124 (100%)	20 (16.1%)	104 (83.9%)	
<i>c-erbB2</i> expression				0.225
0, 1+	121 (100%)	20 (16.5%)	101 (83.5%)	
2+, 3+	47 (100%)	4 (8.5%)	43 (91.5%)	

^a χ^2 test.

immunoreactivity and these clinicopathological parameters could be demonstrated.

CD24 Expression and Patient Survival

Univariate Survival Analysis

In univariate survival analyses, cumulative survival curves were calculated according to the Kaplan-Meier method (Table 3). Differences in survival were assessed with the log-rank test. We analyzed the impact of all of the parameters on overall survival and disease-free survival. The conventional prognostic markers tumor grade, size/pT, nodal status, and disease stage according to Unio Internationale Contra Cancrum reached significance for overall and disease-free survival. Cytoplasmic *CD24* positivity was associated with shorter disease-free survival ($P = 0.0008$) and overall survival ($P = 0.031$), whereas the influence of total *CD24* expression was only significant for shorter disease-free survival ($P = 0.004$). Membranous *CD24* remained insignificant in either analysis. Fig. 2 illustrates the impact of *CD24* expression on survival times.

Survival Analysis in Patient Subgroups. To assess whether *CD24* is a true prognostic marker or a marker of sensitivity to adjuvant therapy, we repeated the aforementioned analyses in the groups of patients who had received either CTX only or TAM with or without CTX. Kaplan-Meier analysis revealed that in both therapy subgroups, total *CD24*-positive tumors were associated with shorter disease-free survival times,

reaching significance in the CTX group ($P = 0.0298$) and a trend ($P = 0.0639$) in the TAM group. For cytoplasmic *CD24*, only in the TAM group could a significantly shorter disease-free survival time be demonstrated (CTX: $P = 0.129$; TAM: $P = 0.0055$). In the accordingly stratified analysis of overall survival times, no significant differences became apparent. In patients who had not received any systemic therapy ($n = 49$), no impact of *CD24* status on either disease-free survival or overall survival was found, which might reflect the few cases.

Significantly shorter disease-free survival times were also found in *CD24*-positive cases in the subgroups of estrogen-positive tumors ($P = 0.0145$) and tumors without *c-erbB2* overexpression ($P = 0.0189$).

Multivariate Survival Analysis

The analysis was based on the Cox regression model to test the influence of each parameter on overall and disease-free survival. In the Cox regression we included *CD24* expression (negative versus positive), tumor grade (G1, G2, and G3), nodal status (negative versus positive), and pT-stage (pT1, pT2, pT3, and pT4). We tested the impact of total *CD24* (Table 4) and cytoplasmic *CD24* (Table 5) on overall and disease-free survival.

Disease-free survival time was significantly dependent on nodal status, tumor grading, and *CD24* expression for cytoplasmic and total *CD24* positivity alike. The number of events (deaths) was insufficient for a valid Cox regression analysis of overall survival. Although we observed relative risk values similar to those of the disease-free survival analysis, these did not reach significance with the exception of pT stage.

In a preliminary multivariate analysis we have tried to evaluate the prognostic significance of *CD24* in different adjuvant therapy subgroups. In this stratified repetition of this multivariate analysis, *CD24* showed only a trend ($P = 0.066$) for disease-free survival and lost its significance for overall survival ($P = 0.171$). This might be based on the few cases in either group and requires a larger study.

DISCUSSION

CD24 has been described in B-cell development and B-cell neoplasia, in the developing pancreas and brain, and in regenerating muscle, keratinocytes, renal tubules, and a large variety of malignant tumors (12, 13, 18–30, 32–34). In this immunohistochemistry-based study we describe the expression of *CD24* protein in breast cancer. Basically, we can confirm the findings of Fogel *et al.* (31), who were the first to describe *CD24* expression of breast cancer in an immunohistochemistry study based on frozen sections. They reported an apical membranous *CD24* immunoreactivity in benign ducts and an additional cytoplasmic staining in invasive carcinomas, and described *CD24* expression as a marker of breast cancer. We used a commercially available monoclonal *CD24* antibody that is applicable to paraffinized tumor tissue and, thus, made a comprehensive analysis of clinically characterized archive material possible. The specificity of the antibody was additionally ascertained by cytofluorographic analysis using a described *CD24*-transfected cell line (31).

Our study suggests *CD24* protein expression as a new

Table 3 Univariate survival analysis (Kaplan-Meier): survival times of all patients with breast cancer according to clinicopathological factors and cytoplasmic or total CD24 expression

Characteristic	Overall survival				Disease-free survival			
	No. of cases	No. of events	5-year survival rate (\pm SE)	<i>P</i>	No. of cases	No. of events	5-year nonprogression rate (\pm SE)	<i>P</i>
Total CD24 expression				0.286				0.004
Negative	31	3	87.1 \pm 6.9		31	3	89.0 \pm 6.0	
Positive	170	31	85.5 \pm 3.1		170	65	60.4 \pm 4.3	
Cytoplasmic CD24				0.031				0.0008
Negative	48	3	91.9 \pm 4.5		48	7	88.3 \pm 4.9	
Positive	153	31	83.8 \pm 3.4		153	61	57.0 \pm 4.7	
Age				0.834				0.111
<60 years	105	17	88.5 \pm 3.5		105	41	57.2 \pm 5.7	
\geq 60 years	96	17	82.9 \pm 4.4		96	27	72.5 \pm 5.1	
Histology				0.503				0.348
Ductal	163	29	85.1 \pm 3.1		163	58	64.2 \pm 4.3	
Lobular	21	2	93.8 \pm 6.1		21	4	73.3 \pm 11.8	
pT stage				0.0004				0.0001
pT1	127	10	94.4 \pm 2.3		127	32	75.9 \pm 4.3	
pT2	58	18	72.3 \pm 7		58	31	38.1 \pm 7.9	
pT3/4	16	10	65.6 \pm 12.6		16	5	65.6 \pm 12.6	
Nodal status				0.001				0.0004
pN0	94	5	94.6 \pm 2.7		94	19	78.8 \pm 4.8	
pN1+	104	28	78.0 \pm 4.5		104	47	52.7 \pm 5.7	
UICC stage				0.0042				0.0017
I	96	6	94.7 \pm 2.6		96	20	78.3 \pm 4.8	
II	74	18	81.2 \pm 5.2		74	35	50.4 \pm 6.9	
III	31	10	65.4 \pm 9.7		31	13	55.7 \pm 9.9	
Histological grade				0.026				0.0001
G1	47	5	90.0 \pm 5.5		47	8	80.1 \pm 7.0	
G2	104	15	89.2 \pm 3.4		104	34	69.6 \pm 5.1	
G3	50	14	74.7 \pm 6.7		50	26	36.6 \pm 8.9	
Estrogen receptor				0.150				0.072
Negative	57	14	80.4 \pm 5.7		57	25	54.6 \pm 7.6	
Positive	124	17	87.1 \pm 3.5		124	37	69.3 \pm 4.8	
c-erbB2 expression				0.507				0.264
0, 1+	121	21	85.6 \pm 3.6		121	39	69.5 \pm 5	
2+, 3+	47	8	84.8 \pm 5.8		47	18	54.9 \pm 8.1	

prognostic marker in breast cancer that retained its prognostic impact on disease-free survival even in a multivariate analysis. Because the majority of breast cancers is *CD24* positive, this new information is especially valuable for patients with *CD24*-negative tumors, who appear to carry a very low risk of tumor progression, as shown in Kaplan-Meier curves (Fig. 2). Interestingly, this prognostic impact of *CD24* is especially significant in estrogen-positive or *c-erbB2*-negative (0, 1+) tumors, subgroups supposed to carry a more favorable prognosis under current therapy regimens. Similarly to our recent study on ovarian cancer, the prognostic value of *CD24* positivity appeared to be strongest when the analysis concentrated on the cytoplasmic *CD24* staining. This is a staining quality we predominantly observed in malignancy and, therefore, might reflect a pathological condition.

A limitation of our study and a general problem of comparable studies are the few patients who had not received any adjuvant therapy. Therefore, we feel it is virtually impossible on the ground of our data to decide whether the prognostic impact of *CD24* expression is really restricted to patients with adjuvant therapy. Clearly, a larger study cohort is needed to address the question of whether *CD24* is more of a true prognostic marker of patient outcome or a predictive marker of sensitivity to therapy.

We found the immunostainings with the *CD24* antibody Ab-2 easy to analyze and reproducible. However, it should be noted that even in experienced hands, the intensity of immunostainings tends to be variable in different laboratories. An elegant way to standardize immunostainings would be to introduce controls, e.g., cell lines with a defined *CD24* protein expression status, to accompany each staining set. We are currently screening various cell lines for their *CD24* status to establish suitable controls.

How does *CD24* positivity of the primary tumor lead to an increased rate of local recurrences? In general, local recurrences result either from residual gross tumor that has been overlooked by the surgeon and the pathologist or from local micrometastases that escaped the scrutiny of both. A correlation of *CD24*-positive tumors with positive resection margins is rather unlikely; therefore, we hypothesize that local tumor cell spread is the most likely explanation for the shortened disease-free survival time. There is *in vitro* evidence for a prometastatic role of *CD24* in human tumor cells based on its function as a ligand to P-selectin (35–39). P-selectin is expressed by activated endothelial cells and platelets, and it plays an important role in marginal adhesion and migration of cells. Possibly, *CD24* positive tumor cells can spread more easily because of their capacity to either form thrombi with activated platelets or to adhere to

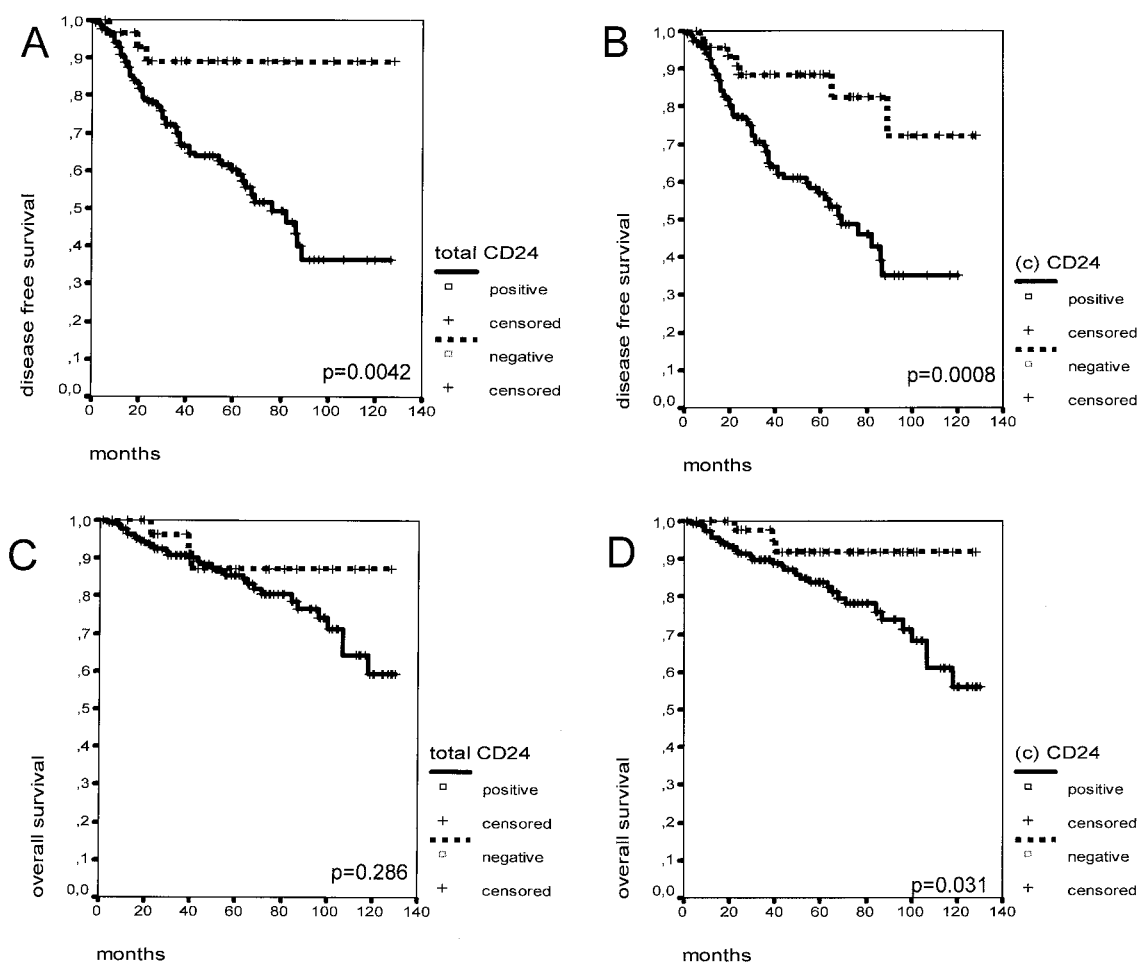


Fig. 2 Kaplan-Meier curves for overall survival of tumor grading, stage, and *CD24*. Impact of total (A) and cytoplasmic (B) *CD24* expression on disease-free survival, and of total (C) and cytoplasmic (D) *CD24* expression on overall survival.

Table 4 Results of the Cox regression model, conventional parameters, and total *CD24*

	Overall survival (33 events)			Disease-free survival (66 events)		
	Relative ratio	95% Confidence interval	<i>P</i>	Relative ratio	95% Confidence interval	<i>P</i>
<i>CD24</i> (total)	1.713	0.516–5.689	0.379	3.838	1.199–12.288	0.023
pT stage	1.479	0.984–2.224	0.06	0.909	0.639–1.293	0.596
Nodal status	2.451	0.833–7.217	0.104	2.077	2.077	0.021
Grading	1.507	0.877–2.590	0.137	1.849	1.253–2.729	0.002

endothelia in the bloodstream, which has been demonstrated for *CD24*-expressing breast cancer cells (38). Moreover, *CD24* positivity might induce an increased invasiveness of a tumor as suggested by *in vivo* data from Senner *et al.* (29), who reported a locally more aggressive behavior of *CD24*-positive gliomas in a mouse model. However, this hypothesis is not in concordance with the findings of Schindelmann *et al.* (42), who screened cell lines of invasive breast cancer and noninvasive breast cells for differential gene expression using cDNA microarrays and reported a significantly down-regulated *CD24* expression in invasive tumor cell lines.

CD24 expression has been associated with an adverse prognosis in ovarian cancer, non-small cell lung cancer, and prostate cancer (11–13). The confirmation of this finding in breast cancer underscores the importance of *CD24* in the disease progression of human carcinomas. Additional confirmational studies are currently being conducted to verify our results to establish *CD24* as a molecular prognostic marker in breast cancer. This might help the clinician to individualize the surveillance scheme/therapy, for example, favoring a more aggressive regimen in strongly *CD24*-positive tumors. In addition, transplantation-associated B-cell proliferative syndrome had

Table 5 Results of the Cox regression model, conventional parameters, and cytoplasmic CD24

	Overall survival (33 events)			Disease free survival (66 events)		
	Relative ratio	95% Confidence interval	P	Relative ratio	95% Confidence interval	P
CD24 (cytoplasmic)	3.089	0.917–10.412	0.069	2.652	1.190–5.909	0.017
pT stage	1.554	1.030–2.345	0.036	0.916	0.642–1.309	0.631
Nodal status	2.369	0.808–6.943	0.116	2.148	1.157–3.990	0.016
Grading	1.327	0.754–2.338	0.327	1.679	1.124–2.507	0.011

been treated with i.v. administration of CD21- and CD24-specific antibodies by Fischer *et al.* (43) and others (44, 45). Possibly, this new therapeutic option might deserve consideration in CD24-positive breast carcinomas as well.

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REFERENCES

- Arbeitsgemeinschaft Bevölkerungsbezogener Krebsregister in Deutschland. Krebs in Deutschland. 3. erweiterte, aktualisierte Ausgabe, Saarbrücken, 2002.
- Jemal, A., Thomas, A., Murray, T., and Thun, M. Cancer statistics, 2002. *CA Cancer J. Clin.*, 52: 23–47, 2002.
- Hayes, D. F., Isaacs, C., and Stearns, V. Prognostic factors in breast cancer: current and new predictors of metastasis. *J. Mammary Gland. Biol. Neoplasia*, 6: 375–392, 2001.
- Mori, I., Yang, Q., and Kakudo, K. Predictive and prognostic markers for invasive breast cancer. *Pathol. Int.*, 52: 186–194, 2002.
- Poremba, C., Heine, B., Diallo, R., Heinecke, A., Wai, D., Schaefer, K. L., Braun, Y., Schuck, A., Lanvers, C., Bankfalvi, A., Kneif, S., Torhorst, J., Zuber, M., Kochli, O. R., Mross, F., Dieterich, H., Sauter, G., Stein, H., Fogt, F., and Boecker, W. Telomerase as a prognostic marker in breast cancer: high-throughput tissue microarray analysis of hTERT and hTR. *J. Pathol.*, 198: 181–189, 2002.
- Yousef, G. M., Scorilas, A., Kyriakopoulou, L. G., Rendl, L., Diamandis, M., Ponzzone, R., Biglia, N., Giai, M., Roagna, R., Sismondi, P., and Diamandis, E. P. Human kallikrein gene 5 (KLK5) expression by quantitative PCR: an independent indicator of poor prognosis in breast cancer. *Clin. Chem.*, 48: 1241–1250, 2002.
- Duffy, M. J. Urokinase plasminogen activator and its inhibitor, PAI-1, as prognostic markers in breast cancer: from pilot to level 1 evidence studies. *Clin. Chem.*, 48: 1194–1197, 2002.
- Nakopoulou, L., Giannopoulou, I., Stefanaki, K., Panayotopoulou, E., Tsimpa, I., Alexandrou, P., Mavrommatis, J., Katsarou, S., and Davaris, P. Enhanced mRNA expression of tissue inhibitor of metalloproteinase-1 (TIMP-1) in breast carcinomas is correlated with adverse prognosis. *J. Pathol.*, 197: 307–313, 2002.
- Rudland, P. S., Platt-Higgins, A., El-Tanani, M., De Silva Rudland, S., Barraclough, R., Winstanley, J. H., Howitt, R., and West, C. R. Prognostic significance of the metastasis-associated protein osteopontin in human breast cancer. *Cancer Res.*, 62: 3417–3427, 2002.
- Spizzo, G., Obrist, P., Ensinger, C., Theurl, I., Dunser, M., Ramoni, A., Gunsilius, E., Eibl, G., Mikuz, G., and Gastl, G. Prognostic significance of Ep-CAM AND Her-2/neu overexpression in invasive breast cancer. *Int. J. Cancer*, 98: 883–888, 2002.
- Kristiansen, G., Denkert, C., Schlüns, K., Dahl, E., Pilarsky, C., and Hauptmann, S. CD24 is expressed in ovarian cancer and is a new independent prognostic marker of patient survival. *Am. J. Pathol.*, 161: 1215–1221, 2002.
- Kristiansen, G., Schlüns, K., Yongwei, Y., Denkert, C., Dietel, M., and Petersen, I. CD24 is an independent prognostic marker of survival in non-small cell lung cancer patients. *Br. J. Cancer*, 88: 231–236, 2003.
- Kristiansen, G., Pilarsky, C., Pervan, J., Stürzebecher, B., Stephan, C., Jung, K., Loening, S., Rosenthal, A., and Dietel, M. CD24 expression is a significant predictor of PSA relapse and poor prognosis in low grade or organ confined prostate cancer. *Prostate*, in press, 2003.
- Pirucello, S. J., and LeBien, T. W. The human B cell-associated antigen CD24 is a single chain sialoglycoprotein. *J. Immunol.*, 136: 3779–3784, 1986.
- Fischer, G. F., Majdic, O., Gadd, S., and Knapp, W. Signal transduction in lymphocytic and myeloid cells via CD24, a new member of phosphoinositol-anchored membrane molecules. *J. Immunol.*, 144: 638–641, 1990.
- Lavabre-Bertrand, T., Duperray, C., Brunet, C., Poncelet, P., Exbrayat, C., Bourquard, P., Lavabre-Bertrand, C., Brochier, J., Navarro, M., and Janossy, G. Quantification of CD24 and CD45 antigens in parallel allows a precise determination of B-cell maturation stages: relevance for the study of B-cell neoplasias. *Leukemia (Baltimore)*, 8: 402–408, 1994.
- Akashi, T., Shirasawa, T., and Hirokawa, K. Gene expression of CD24 core polypeptide molecule in normal rat tissues and human tumor cell lines. *Virchows Arch.*, 425: 399–406, 1994.
- Poncet, C., Frances, V., Gristina, R., Scheiner, C., Pellissier, J. F., and Figarella-Branger, D. CD24, a glycosylphosphatidylinositol-anchored molecules is transiently expressed during the development of human central nervous system and is a marker of human neural cell lineage tumors. *Acta Neuropathol. (Berl)*, 91: 400–408, 1996.
- Cram, D. S., McIntosh, A., Oxbrow, L., Johnson, A. M., and DeAizpurua, H. J. Differential mRNA display analysis of two related but functionally distinct rat insulinoma (RIN) cell lines: identification of CD24 and its expression in the developing pancreas. *Differentiation*, 64: 237–246, 1999.
- Shirasawa, T., Akashi, T., Sakamoto, K., Takahashi, H., Maruyama, N., and Hirokawa, K. Gene expression of CD24 core peptide molecule in developing brain and developing non-neural tissues. *Dev. Dyn.*, 198: 1–13, 1993.
- Figarella-Branger, D., Moreau, H., Pellissier, J. F., Bianco, N., and Rougon, G. CD24, a signal-transducing molecule expressed on human B lymphocytes, is a marker for human regenerating muscle. *Acta Neuropathol. (Berl)*, 86: 275–284, 1993.
- Redondo, P., Garcia-Foncillas, J., Okroujnov, I. de Felipe, I., and Quintanilla, E. CD24 expression on human keratinocytes. *Exp. Dermatol.*, 7: 175–178, 1998.
- Droz, D., Zachar, D., Charbit, L., Gogusev, J., Crétien, Y., and Iris, L. Expression of the human nephron differentiation molecules in renal cell carcinoma. *Am. J. Pathol.*, 137: 895–905, 1990.
- Pirruccello, S. J., and Lang, M. S. Differential expression of CD24-related epitopes in mycosis fungoides/Sezary syndrome: a potential marker for circulating Sezary cells. *Blood*, 76: 2343–2347, 1990.
- Raife, T. J., Lager, D. J., Kemp, J. D., and Dick, F. R. Expression of CD24 (BA-1) predicts monocytic lineage in acute myeloid leukemia. *Am. J. Clin. Pathol.*, 101: 296–299, 1994.

26. Jackson, D., Waibel, R., Weber, E., Bell, J., and Stahel, R. A. CD24, a signal-transducing molecule expressed on human B cells, is a major surface antigen on small cell lung carcinomas. *Cancer Res.*, *52*: 5264–5270, 1992.
27. Karran, L., Jones, M., Morley, G., van Noorden, S., Smith, P., Lampert, I., and Griffins, B. E. Expression of a B-cell marker, CD24, on nasopharyngeal carcinoma cells. *Int. J. Cancer*, *60*: 562–566, 1995.
28. Huang, L. R., and Hsu, H. C. Cloning and expression of CD24 gene in human hepatocellular carcinoma: a potential early tumor marker gene correlates with p53 mutation and tumor differentiation. *Cancer Res.*, *55*: 4717–4721, 1995.
29. Senner, V., Sturm, A., Baur, I., Schrell, U. H., Distel, L., and Paulus, W. CD24 promotes invasion of glioma cells *in vivo*. *J. Neuro-pathol. Exp. Neurol.*, *58*: 795–802, 1999.
30. Gromova, I., Gromov, P., and Celis, J. E. Identification of true differentially expressed mRNAs in a pair of human bladder transitional cell carcinomas using an improved differential display procedure. *Electrophoresis*, *20*: 241–248, 1999.
31. Fogel, M., Friederichs, J., Zeller, Y., Husar, M., Smirnov, A., Roitman, L., Altevogt, P., and Stoeber, Z. M. CD24 is a marker for human breast carcinoma. *Cancer Lett.*, *143*: 87–94, 1999.
32. Yang, G. P., Ross, D. T., Kuang, W. W., Brown, P. O., and Weigel, R. J. Combining SSH and cDNA microarrays for rapid identification of differentially expressed genes. *Nucleic Acids Res.*, *27*: 1517–1523, 1999.
33. Liu, W., and Vadgama, J. V. Identification and characterization of amino acid starvation-induced CD24 gene in MCF-7 in human breast cancer cells. *Int. J. Oncol.*, *16*: 1049–1054, 2000.
34. Welsh, J. B., Zarrinkar, P. P., Sapinoso, L. M., Kern, S. G., Behling, C. A., Monk, B. J., Lockhart, D. J., Burger, R. A., and Hapton, G. M. Analysis of gene expression profiles in normal and neoplastic ovarian tissue samples identifies candidate molecular markers of epithelial ovarian cancer. *Proc. Natl. Acad. Sci. USA*, *98*: 1176–1181, 2001.
35. Sammar, M., Aigner, S., Hubbe, M., Schirmacher, V., Schachner, M., Vestweber, D., and Altevogt, P. Heat-stable antigen (CD24) as ligand for mouse P-selectin. *Int. Immunol.*, *6*: 1027–1036, 1994.
36. Aigner, S., Ruppert, M., Hubbe, M., Sammar, M., Stoeber, Z., Butcher, E. C., Vestweber, D., and Altevogt, P. Heat stable antigen (mouse CD24) supports myeloid cell binding to endothelial and platelet P-selectin. *Int. Immunol.*, *7*: 1557–1565, 1995.
37. Aigner, S., Stoeber, Z. M., Fogel, M., Weber, E., Zarn, J., Ruppert, M., Zeller, Y., Vestweber, D., Stahel, R., Sammar, M., and Altevogt, P. CD24, a mucin-type glycoprotein, is a ligand for P-selectin on human tumor cells. *Blood*, *89*: 3385–3395, 1997.
38. Aigner, S., Ramos, C. L., Hafezi-Moghadam, A., Lawrence, M. B., Friederichs, J., Altevogt, P., and Ley, K. CD24 mediates rolling of breast carcinoma cells on P-selectin. *FASEB J.*, *12*: 1241–1251, 1998.
39. Friederichs, J., Zeller, Y., Hafezi-Moghadam, A., Grone, H. J., Ley, K., and Altevogt, P. The CD24/P-selectin binding pathway initiates lung arrest of human A125 adenocarcinoma cells. *Cancer Res.*, *60*: 6714–6722, 2000.
40. Sobin, L. H., and Wittekind, C. (eds.). *TNM Classification of Malignant Tumours*, Ed. 5. New York: Wiley-Liss Inc., pp. 123–130, 1997.
41. Elston, C. W., and Ellis, I. O. 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*, *19*: 403–410, 1991.
42. Schindelmann, S., Windisch, J., Grundmann, R., Kreienberg, R., Zeillinger, R., and Deissler, H. Expression profiling of mammary carcinoma cell lines: correlation of *in vitro* invasiveness with expression of CD24. *Tumor Biol.*, *23*: 139–145, 2002.
43. Fischer, A., Blanche, S., Le Bidois, J., Bordigoni, P., Garnier, J. L., Niaudet, P., Morinet, F., Le Deist, F., Fischer, A. M., and Griscelli, C. Anti-B-cell monoclonal antibodies in the treatment of severe B-cell lymphoproliferative syndrome following bone marrow and organ transplantation. *N. Engl. J. Med.*, *324*: 1451–1456, 1991.
44. Benkerrou, M., Jais, J. P., Leblond, V., Durandy, A., Sutton, L., Bordigoni, P., Garnier, J. L., Le Bidois, J., Le Deist, F., Blanche, S., and Fischer, A. Anti-B-cell monoclonal antibody treatment of severe post-transplant B-lymphoproliferative disorder: prognostic factors and long-term outcome. *Blood*, *92*: 3137–3147, 1998.
45. Garnier, J. L., Stevenson, G., Blanc-Brunat, N., Touraine, J. L., Milpied, N., Leblond, V., and Blay, J. Y. Treatment of post-transplant lymphomas with anti-B-cell monoclonal antibodies. *Recent Results Cancer Res.*, *159*: 113–122, 2002.