High $p27^{Kip1}$ Expression Predicts Superior Relapse-Free and Overall Survival for Premenopausal Women With Early-Stage Breast Cancer Receiving Adjuvant Treatment With Tamoxifen Plus Goserelin

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Purpose: **To determine the predictive value of $p27^{Kip1}$ in premenopausal women with early-stage hormone receptor–positive breast cancer.**

Patients and Methods: **We retrospectively examined tumor specimens from 512 patients with breast cancer who were enrolled onto Austrian Breast and Colorectal Cancer Study Group (ABCSG) Trial 5. In this trial, premenopausal, hormone receptor–positive breast cancer patients with stage I and II disease were randomly assigned to receive either 5 years of tamoxifen plus 3 years of goserelin or six cycles of cyclophosphamide, methotrexate, and fluorouracil. $p27^{Kip1}$ expression was assessed by immunohistochemistry, and its association with clinical outcome was determined. Statistical analyses were performed to test for interaction between $p27^{Kip1}$ status and treatment.**

Results: **High $p27^{Kip1}$ expression (nuclear $p27^{Kip1}$ staining in ≥ 50% of tumor cells) independently predicted superior relapse-free survival (RFS) and overall survival (OS) in both the total study population (RFS: relative risk [RR], 0.53; 95% CI, 0.34 to 0.82; *P* = .004; OS: RR, 0.29; 95% CI, 0.15 to 0.58; *P* < .001) and patients treated with combination endocrine therapy (RFS: RR, 0.32; 95% CI, 0.16 to 0.63; *P* = .001; OS: RR, 0.16; 95% CI, 0.05 to 0.53; *P* = .003). The interaction between $p27^{Kip1}$ expression and treatment was statistically significant for RFS (*P* = .04) but not for OS (*P* = .27).**

Conclusion: **High $p27^{Kip1}$ expression was an independent predictor of responsiveness to hormonal therapy and thus may be useful for the selection of premenopausal women with early-stage hormone receptor–positive breast cancer for adjuvant combination endocrine therapy.**

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THE DEFINITION of accurate markers for the selection of the appropriate adjuvant therapy for patients with early-stage breast cancer would improve efficacy and avoid unnecessary toxicity and long-term side effects in patients not responsive to the selected adjuvant treatment. One of the most promising molecular markers currently being studied is the cell cycle regulator $p27^{Kip1}$.

Progression from G1 to the S phase of the cell cycle is regulated by the formation of cyclin/cyclin-dependent kinase (CDK) complexes.[1] CDK activity is inhibited by CDK inhibitory proteins, including the Cip/Kip family members $p21^{Waf1/Cip1}$, $p27^{Kip1}$, and $p57^{Kip2}$.[2,3] These proteins interact with complexes containing cyclin D, E, and A,[4-6] and recent data suggest they exert both positive and negative regulation of CDK activity at G1/S transition.[7-9] Various functions have been attributed to $p27^{Kip1}$, including promotion of apoptosis[10,11] and regulation of drug resistance.[12] In addition, decreased expression of $p27^{Kip1}$ is associated with poor clinical outcome in a variety of malignant diseases. Various groups have studied $p27^{Kip1}$ expression in primary breast cancer. Whereas $p27^{Kip1}$ protein reduction was a strong independent prognostic factor for disease-free survival (DFS) and overall survival (OS)[13-18] in most studies, others did not confirm these findings.[19,20]

Preclinical data suggest that $p27^{Kip1}$ is an essential mediator of cell cycle arrest by tamoxifen and other antiestrogenic drugs. Results of a recent study suggest that, in addition to the estrogen receptor, a breast cancer cell must express functional $p27^{Kip1}$ for tamoxifen to mediate its cytostatic effects.[21] This observation raises the hypothesis that deregulation and loss of $p27^{Kip1}$ may contribute to both hormone independence and tamoxifen resistance in breast cancer.

The present study was designed to determine whether $p27^{Kip1}$ could be used as a marker to identify a subgroup of patients more likely to benefit from adjuvant combination endocrine therapy likely than others. For this study, we have chosen patients enrolled onto the Austrian Breast and Colorectal Cancer Study Group (ABCSG) Trial 5, a prospective randomized trial comparing the efficacy of a combination endocrine treatment with cyclophosphamide, methotrexate, and fluorouracil (CMF) chemotherapy.

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PATIENTS AND METHODS

ABCSG Trial 5

The objective of ABCSG Trial 5 was to compare the efficacy of a combination endocrine treatment with standard CMF chemotherapy.[22] From December 1990 to October 1999, a total of 1,099 patients were entered, of whom 1,034 patients were assessable for the final analysis. Patients were stratified by tumor size, number of involved lymph nodes, type of curative surgery, tumor grade, and hormone receptor status. Patients with hormone receptor–positive breast cancer were randomly assigned to receive either five years of tamoxifen (Nolvadex; AstraZeneca Pharmaceuticals, Wilmington, DE) plus three years of goserelin (Zoladex; AstraZeneca Pharmaceuticals) or six cycles of CMF. None of the trial participants received tamoxifen after CMF treatment. The results of this study suggest that combination endocrine therapy is more effective than CMF in the adjuvant treatment of premenopausal patients with stage I or II breast cancer. The results of ABCSG Trial 5 are reported elsewhere.[22]

All patients registered onto ABCSG Trial 5 were eligible for entry to the laboratory study, and the major participating centers were requested to provide tumor blocks of their patients.

Treatment Regimens

CMF was administered intravenously for six cycles, days 1 and 8, recycled on day 28, at the following doses: cyclophosphamide 600 mg/m^2, methotrexate 40 mg/m^2, and fluorouracil 600 mg/m^2. Goserelin was given subcutaneously at 3.6 mg per injection every 28 days for 3 years (39 injections). Tamoxifen was administered at 20 mg orally once a day for 5 years.

Immunohistochemistry

All tumor specimens were obtained at the time of surgery before the adjuvant therapy. Formalin-fixed, paraffin-embedded blocks from the primary breast lesions were used for p27^{Kip1} immunostaining. A hematoxylin and eosin–stained slide was prepared from each block and used for pathologic confirmation of the presence of invasive breast cancer. All slides were reviewed by a pathologist (M.R.) who was blinded to clinical outcome. Immunohistochemical analysis reported in this study was carried out in a single laboratory (Clinical Division of Oncology, Department of Medicine I).

Tissue sections of 4 μm thickness were prepared, mounted on poly-*L*-lysine-coated slides, deparaffinized, and rehydrated with distilled water. Endogenous peroxidase activity was blocked by incubation in 0.06% hydrogen peroxide for 10 minutes at room temperature. After boiling for 10 minutes in 10 mmol/L citrate buffer (pH 6.0) for antigen retrieval, the tissues were preincubated for 20 minutes in normal serum (1:50; DakoCytomation, Glostrup, Denmark) before a 60-minute incubation with the anti p27^{Kip1} monoclonal antibody (clone 57; antibody used at 1.25 μg/mL; Transduction Laboratories, Lexington, KY). Antibody binding was detected by the avidin-biotin-peroxidase method. Bound peroxidase was developed with 3,3′-diaminobenzidine (DakoCytomation). The slides were counterstained with Mayer's hemalum and mounted with Aquatex (Merck, Darmstadt, Germany). All washes were performed in phosphate-buffered saline (pH 7.4).

Expression of p27^{Kip1} in normal epithelial cells and small lymphocytes was used as internal positive control of immunostaining.[14] In addition, negative controls without the primary antibody were performed as described above. Staining of tumor cells was examined independently by two observers (G.P., M.R.) without prior knowledge of the clinical outcome of the patients and the concordance of their evaluation was high. To explore the level of concordance between the two observers in greater detail, kappa statistics were used to assess interobserver reliability, and the observed kappa ratio was 0.91 (data not shown). The rare discrepant cases were reassessed together by both investigators using a double-headed microscope, and a consensus was reached. All invasive tumor cells on each slide were evaluated. Interpretation of the results was limited to the invasive portion of

Table 1. Comparison of ABCSG Trial 5 and p27^{Kip1} Study

Variable	Percentage of Patients in Endocrine Therapy Arm		Percentage of Patients in Chemotherapy Arm	
	p27^{Kip1} Study (n = 251)	ABCSG Trial 5 (n = 511)	p27^{Kip1} Study (n = 261)	ABCSG Trial 5 (n = 523)
Age, years				
< 35	7	7	6	7
≥ 35	93	93	94	93
Pathologic tumor size				
pT1	58	57	61	58
pT2	38	40	36	39
pT3	4	4	3	4
Lymph node status				
Negative	56	51	48	50
Positive: 1–3 lymph nodes	30	34	38	35
Positive: 4–10 lymph nodes	13	12	12	13
Positive: > 10 lymph nodes	1	3	2	2
Tumor grade				
G1, G2	68	72	69	72
G3	32	28	31	28
Hormone receptor status				
ER-negative	8	6	6	7
ER-positive	63	68	68	69
Strongly ER-positive	29	25	26	24
PgR-negative	9	9	12	11
PgR-positive	46	48	54	54
Strongly PgR-positive	45	43	34	34
Relapses	18	17	23	21
Deaths	6	8	8	10

Abbreviations: ABCSG, Austrian Breast and Colorectal Cancer Study Group; ER, estrogen receptor; PgR, progesterone receptor.

Table 2. Association of p27^{Kip1} With Classical Prognostic Variables and Adjuvant Treatment

Variable	Total No. of Patients (N = 512)	Low p27^{Kip1} (n = 99)		High p27^{Kip1} (n = 413)		*P*
		No. of Patients	%	No. of Patients	%	
Age, years						
< 35	33	8	8	25	6	.5
≥ 35	479	91	92	388	94	
Pathologic tumor size						
pT1	304	53	54	251	61	.4
pT2	189	42	42	147	36	
pT3	19	4	4	15	3	
Lymph node status						
Negative	267	55	56	212	51	.5
Positive: 1–3 lymph nodes	174	33	33	141	34	
Positive: 4–10 lymph nodes	63	11	11	52	13	
Positive: > 10 lymph nodes	8	0	0	8	2	
Tumor grade						
G1	59	6	6	53	13	.15
G2	292	58	59	234	57	
G3	161	35	35	126	30	
Hormone receptor status						
ER-negative	36	10	10	26	6	.13
ER-positive	337	69	70	268	65	
Strongly ER-positive	139	20	20	119	29	
PgR-negative	54	16	16	38	9	.13
PgR-positive	255	47	48	208	51	
Strongly PgR-positive	203	36	36	167	40	
Adjuvant treatment						
Endocrine therapy	251	50	51	201	49	.7
Chemotherapy	261	49	49	212	51	

Abbreviations: ER, estrogen receptor; PgR, progesterone receptor.

the tumor, and only nuclear staining was scored as positive. At least 200 tumor cells per case were evaluated and the result expressed as the percentage of $p27^{Kip1}$-labeled nuclei.

Comparisons of $p27^{Kip1}$ expression with clinical parameters and outcome were performed with $p27^{Kip1}$ expression as a dichotomized variable classified as low (nuclear $p27^{Kip1}$ staining in $<$ 50% of tumor cells) or high (nuclear $p27^{Kip1}$ staining in $\geq$ 50% of tumor cells). This cutoff was based on previously published reports demonstrating the prognostic significance of $p27^{Kip1}$ expression in breast carcinomas.[14-19]

Statistical Analysis

Associations of $p27^{Kip1}$ expression with age, tumor size, lymph node status, tumor grade, estrogen receptor (ER), and progesterone receptor (PgR) were assessed by the χ^2 test. Survival probabilities were estimated with the Kaplan-Meier product limit method.[23] Survival time was defined as the period between the time of randomization and death (OS) or the period between the time of randomization and documented relapse (relapse-free survival [RFS]). Survival times of patients still alive were censored with the date of the last follow-up. Differences between survival curves were analyzed by the log-rank test. To describe the unadjusted effects of covariates on OS and RFS, univariate Cox proportional hazards regression models were used. Multiple Cox models were used to assess the independent effects of $p27^{Kip1}$ expression on OS and RFS.[24] All *P* values are results of two-sided tests. The SPSS 10.0 statistical software (SPSS Inc, Chicago, IL) was used for calculations.

RESULTS

Tumor blocks of 512 patients were available for $p27^{Kip1}$ immunohistochemical studies. The main clinical and laboratory parameters of these patients compared with all 1,034 patients enrolled in ABCSG Trial 5 are summarized in Table 1. Patients in each treatment group were balanced by variables listed in Table 1 and were also similar to those in the parent clinical trial. Therefore, the present study cohort was representative of the original study population enrolled in ABCSG Trial 5.

$p27^{Kip1}$ immunostaining was usually nuclear and ranged from 0% to 100% (median, 70%) of the breast cancer cells. In some specimens, nuclear and cytoplasmic staining patterns were observed, but only nuclear staining was scored as positive. For comparisons of $p27^{Kip1}$ expression with clinical parameters, $p27^{Kip1}$ expression was used as a dichotomized variable classified as either low (nuclear $p27^{Kip1}$ staining in $<$ 50% of tumor cells) or high (nuclear $p27^{Kip1}$ staining in $\geq$ 50% of tumor cells). High $p27^{Kip1}$ expression was observed in 413 patients (81%). The proportion of high $p27^{Kip1}$ expression was well balanced between the two treatment arms. High $p27^{Kip1}$ expression was observed in 49% of the patients randomly assigned to the combination endocrine treatment arm and in 51% of the patients randomly assigned to the chemotherapy arm ($P = .7$; Table 2).

Clinicopathologic characteristics of the studied population and its association with $p27^{Kip1}$ status are summarized in Table 2. Patients with low or high $p27^{Kip1}$ expression did not differ significantly in age, tumor size, lymph node status, tumor grade, or hormone receptor status.

The median follow-up time of the total study population was 5.5 years, and the maximum follow-up time was 9.5 years. One hundred five patients (20.5%) relapsed (29 patients with low expression and 76 patients with high $p27^{Kip1}$ expression; $P = .016$), and 37 patients(7%) died as a result of cancer (14 patients with low and 23 patients with high $p27^{Kip1}$ expression; $P = .003$). The 5-year RFS and OS rates were 80% and 93%, respectively, for the studied population. Clinical parameters—age, tumor size, lymph node

Table 3. Cox Proportional Hazards Regression Analyses for Relapse-Free and Overall Survival in All 512 Patients

	Relapse-Free Survival					
	Univariate Analysis			Multivariate Analysis		
Variable	RR	95% CI	*P*	RR	95% CI	*P*
Age, years	0.25	0.15 to 0.42	$<$ .001	0.23	0.13 to 0.40	$<$ .001
Tumor size	2.49	1.82 to 3.42	$<$ .001	2.01	1.47 to 2.75	$<$ .001
Lymph node status	2.00	1.62 to 2.48	$<$ .001	1.85	1.47 to 2.35	$<$ .001
Tumor grade	1.33	0.97 to 1.82	.08	1.15	0.83 to 1.59	.4
ER	1.00	0.71 to 1.40	.99	0.85	0.59 to 1.23	.4
PgR	0.72	0.54 to 0.96	.03	0.74	0.55 to 0.98	.04
Treatment	0.73	0.49 to 1.07	.1	0.69	0.47 to 1.02	.06
$p27^{Kip1}$	0.58	0.38 to 0.89	.01	0.53	0.34 to 0.82	.004
	Overall Survival					
	Univariate Analysis			Multivariate Analysis		
Variable	RR	95% CI	*P*	RR	95% CI	*P*
Age, years	0.50	0.19 to 1.30	.15	0.37	0.14 to 0.99	.049
Tumor size	2.46	1.51 to 4.02	$<$ .001	1.73	1.05 to 2.85	.03
Lymph node status	2.51	1.77 to 3.58	$<$ .001	2.27	1.50 to 3.41	$<$ .001
Tumor grade	1.79	1.03 to 3.09	.04	1.40	0.79 to 2.48	.3
ER	0.95	0.54 to 1.68	.86	0.91	0.49 to 1.70	.8
PgR	0.63	0.39 to 1.01	.06	0.73	0.46 to 1.18	.2
Treatment	0.70	0.36 to 1.36	.29	0.81	0.41 to 1.59	.5
$p27^{Kip1}$	0.35	0.18 to 0.69	.002	0.29	0.15 to 0.58	$<$ .001

NOTE. Variables were coded as follows: Age, $<$ 35 years or $\geq$ 35 years; tumor size, pT1, pT2, or pT3; lymph node status, 0, 1–3, 4–10, or $>$ 10 axillary lymph node metastases; tumor grade, G1, G2, or G3; ER, negative, positive, or strongly positive; PgR, negative, positive, or strongly positive; treatment, combination endocrine therapy or chemotherapy; $p27^{Kip1}$, $<$ 50% or $\geq$ 50%.

Abbreviations: RR, relative risk; ER, estrogen receptor; PgR, progesterone receptor.

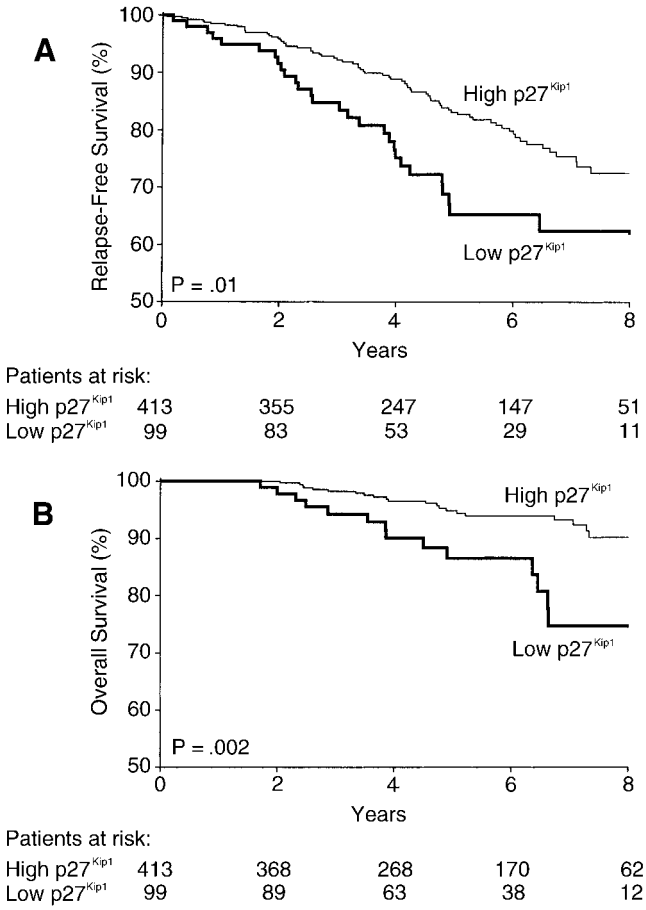

Fig 1. Kaplan-Meier plots for (A) relapse-free survival and (B) overall survival. Survival data based on $p27^{Kip1}$ expression are shown. Patients with low $p27^{Kip1}$ expression had significantly shorter relapse-free survival and overall survival than patients with high $p27^{Kip1}$ expression.

status, PgR, and $p27^{Kip1}$ expression—were significantly associated with relapse-free survival, as determined by univariate analysis (Table 3). Tumor size, lymph node status, tumor grade, and $p27^{Kip1}$ expression were significantly associated with OS as well (Table 3). Patients with high $p27^{Kip1}$ expression had significantly longer RFS and OS times than those with low $p27^{Kip1}$ expression (Fig 1A and B). The Kaplan-Meier estimate of the 5-year RFS rate was 83% in patients with high $p27^{Kip1}$ expression, compared with 65% in patients with low $p27^{Kip1}$ expression ($P = .01$). The 5-year OS rate was 95% for patients with high $p27^{Kip1}$ expression and 87% for those with low $p27^{Kip1}$ expression ($P = .002$). By multivariate Cox regression analyses, high $p27^{Kip1}$ expression was identified as an independent predictor for superior RFS (relative risk [RR], 0.53; 95% CI, 0.34 to 0.82; $P = .004$) and overall survival (RR, 0.29; 95% CI, 0.15 to 0.58; $P < .001$; Table 3).

Because preclinical studies suggested that $p27^{Kip1}$ is essential for responsiveness of breast cancer cells to antiestrogen therapies, we determined RRs of RFS and OS for patients treated with combination endocrine therapy (Table 4). For those patients who received combination endocrine therapy, $p27^{Kip1}$ was found to be an independent predictor of RFS (RR, 0.32; 95% CI, 0.16 to 0.63; $P = .001$) and OS (RR, 0.16; 95% CI, 0.05 to 0.53; $P = .003$). For patients who received chemotherapy, $p27^{Kip1}$ did not predict RFS or OS (data not shown).

To further assess the role of $p27^{Kip1}$ as a predictive marker of response to adjuvant therapy, we compared the effectiveness of combination endocrine treatment relative to CMF chemotherapy on the basis of $p27^{Kip1}$ expression. The results of these analyses are shown in Table 5 and Figure 2.

In patients with high $p27^{Kip1}$ expression, endocrine treatment was superior to chemotherapy, although statistical significance was achieved only for RFS but not for OS. The 5-year RFS rate for patients randomly assigned to endocrine therapy was 88%, compared with 78% for patients randomly assigned to chemotherapy ($P = .02$; Fig 2B). Similarly, the 5-year OS rate for patients who received endocrine therapy was 97%, compared with 93% for those treated with chemotherapy ($P = .17$; Fig 2D). To assess whether the modulation of the endocrine treatment by $p27^{Kip1}$ is independent of other variables, relative risks were estimated before and after adjusting for age, tumor size, lymph node status, tumor grade, ER, and PgR. In the cohort of patients with high $p27^{Kip1}$ expression, the adjusted RRs for relapse and death were 0.52 (95% CI, 0.32 to 0.83; $P = .006$) and 0.51 (95% CI, 0.21 to 1.26; $P = .15$), respectively (Table 5). The interaction of $p27^{Kip1}$ expression and treatment was assessed by use of Cox proportional hazards regression models incorporating the following variables: age, tumor size, lymph node status, tumor grade, ER, PgR, treatment, $p27^{Kip1}$ expression, and an

Table 4. Multivariate Cox Proportional Hazards Regression Analyses for Relapse-Free Survival and Overall Survival in Patients Treated With Combination Endocrine Therapy

Variable	Relapse-Free Survival			Overall Survival		
	RR	95% CI	P	RR	95% CI	P
Age, years	0.23	0.11 to 0.50	< .001	0.43	0.11 to 1.65	.2
Tumor size	1.76	1.08 to 2.86	.02	1.66	0.76 to 3.61	.2
Lymph node status	1.50	1.01 to 2.24	.047	1.29	0.60 to 2.78	.5
Tumor grade	1.49	0.92 to 2.41	.1	2.24	0.94 to 5.38	.07
ER	1.05	0.60 to 1.82	.9	1.86	0.72 to 4.82	.2
PgR	0.79	0.51 to 1.21	.3	0.48	0.24 to 0.95	.04
$p27^{Kip1}$	0.32	0.16 to 0.63	.001	0.16	0.05 to 0.53	.003

NOTE. Variables were coded as follows: Age, < 35 years or ≥ 35 years; tumor size, pT1, pT2, or pT3; lymph node status, 0, 1–3, 4–10, or > 10 axillary lymph node metastases; tumor grade, G1, G2, or G3; ER, negative, positive, or strongly positive; PgR, negative, positive, or strongly positive; $p27^{Kip1}$, < 50% or ≥ 50%.

Abbreviations: RR, relative risk; ER, estrogen receptor; PgR, progesterone receptor.

Table 5. Relative Risks for Relapse and Death to Patients Treated With Combination Endocrine Therapy With Tamoxifen Plus Goserelin Relative to Those Treated With CMF by $p27^{Kip1}$ Status

		Low $p27^{Kip1}$ Status			High $p27^{Kip1}$ Status			
End Point	Adjusted*	RR	95% CI	*P*	RR	95% CI	*P*	Interaction,† *P*
RFS	No	1.10	0.52 to 2.29	.81	0.59	0.37 to 0.93	.02	
	Yes	1.05	0.45 to 2.48	.82	0.52	0.32 to 0.83	.006	.04
OS	No	0.89	0.31 to 2.54	.83	0.54	0.23 to 1.30	.17	
	Yes	0.90	0.26 to 3.10	.87	0.51	0.21 to 1.26	.15	.27

Abbreviations: CMF, cyclophosphamide, methotrexate, and fluorouracil; RR, relative risk; RFS, relapse-free survival; OS, overall survival.

*Adjusted analyses control for the effects of age (< 35 years or ≥ 35 years), tumor size (pT1, pT2, pT3), lymph node status (0, 1–3, 4–10, > 10 axillary lymph node metastases), tumor grade (G1, G2, G3), estrogen receptor (negative, positive, or strongly positive), and progesterone receptor (negative, positive, or strongly positive).

†Interaction between $p27^{Kip1}$ expression (low or high) and treatment (combination endocrine therapy with tamoxifen plus goserelin or CMF).

interaction term, the product of treatment and $p27^{Kip1}$ expression. In these analyses, the interaction term was statistically significant for RFS ($P = .04$) but not for OS ($P = .27$). In the group with low $p27^{Kip1}$ expression, RFS and OS rates for the patients within the endocrine treatment arm were not different from those of the patients within the chemotherapy arm. At 5 years, the RFS rate was 62% in the endocrine treatment arm and 70% in the chemotherapy arm ($P = .81$; Fig 2A). The corresponding 5-year OS rates were and 88% and 85%, respectively ($P = .83$; Fig 2C). The adjusted RRs for relapse and death were 1.05 (95% CI, 0.45 to 2.48; $P = .82$) and 0.9 (95% CI, 0.26 to 3.1; $P = .87$), respectively (Table 5). However, the sample sizes in the low-expression group were small and, therefore, the study did not have sufficient statistical power to detect small differences in survival between the two treatment groups. Nevertheless, these results indicate that combination endocrine therapy may be superior to chemotherapy in patients with high $p27^{Kip1}$ expression.

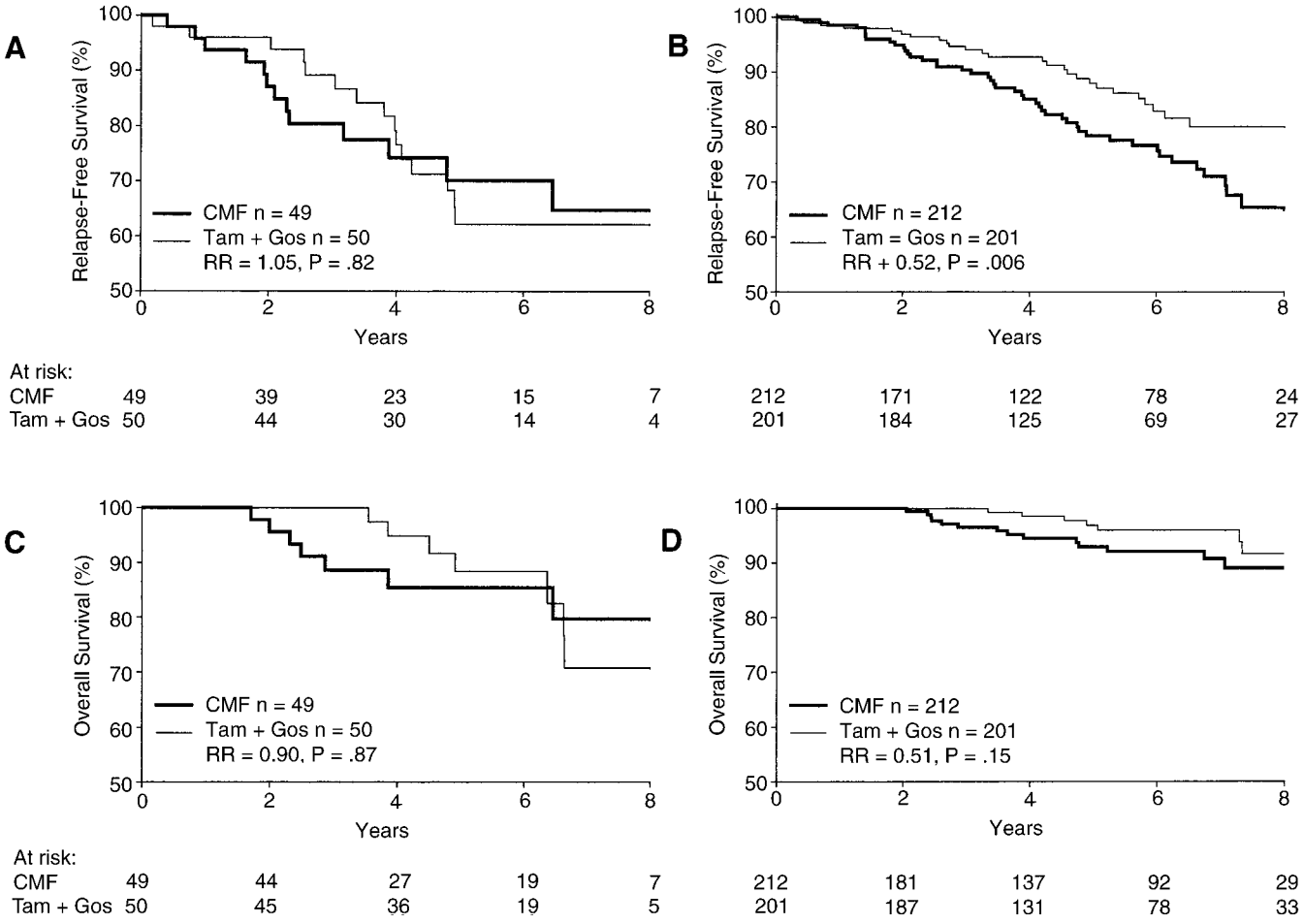

Fig 2. Relapse-free survival (A, B) and overall survival (C, D) for tamoxifen plus goserelin and CMF treatment arms in cohorts with low (A, C) and high (B, D) $p27^{Kip1}$ expression. Relative risks of failure and *P* values shown on each plot are adjusted for age, tumor size, lymph node status, tumor grade, estrogen receptor, and progesterone receptor. CMF, cyclophosphamide, methotrexate, and fluorouracil chemotherapy; Tam, tamoxifen; Gos, goserelin; RR, relative risk.

DISCUSSION

The definition of accurate predictive factors to select the appropriate adjuvant therapy for patients with early-stage breast cancer is of immense importance. So far, the choice of adjuvant therapy is based on patients' lymph node status and hormone receptor status. While there are many molecular markers with potential prognostic value in breast cancer, only few have been evaluated as predictors of response to specific treatments, and most of the currently available data are controversial and/or inconclusive.[25]

In the present study, we examined $p27^{Kip1}$ expression in premenopausal, hormone receptor–positive breast cancer patients with stage I and II disease who were enrolled onto a prospective randomized trial. In this homogenous and well-defined patient population, we observed that low $p27^{Kip1}$ expression is an independent prognostic factor for poor RFS and OS, which is consistent with previous reports.[13-18] More importantly, however, we found an interaction between $p27^{Kip1}$ expression and a specific therapeutic regimen. Patients with high $p27^{Kip1}$ expression who were treated with combination endocrine therapy experienced a 48% relative reduction in relapse rate and a 49% relative decrease in mortality compared with those patients who received CMF. These differences translated to a 10% absolute improvement in RFS and a 4% absolute improvement in OS at 5 years. In contrast, patients with low $p27^{Kip1}$ expression experienced unfavorable outcome regardless of adjuvant combination endocrine therapy or CMF chemotherapy. These differences suggest that combination endocrine therapy may be more active in patients with high $p27^{Kip1}$ expression and that additional or other treatment strategies need to be developed for breast cancer patients with low $p27^{Kip1}$ expression.

Analyses of $p27^{Kip1}$ expression must be performed at the protein level because mutations in the human *p27Kip1* gene are rare,[26-28] in contrast to other cell cycle regulators (such as p16 or p53), and loss of $p27^{Kip1}$ expression is mainly due to increased proteolysis by the ubiquitin-proteasome pathway[29] and not to altered transcription or mRNA stability.[30] The immunohistochemical assay used in the present study can reliably be performed on formalin-fixed, paraffin-embedded tumor specimens and is a simple and appropriate detection method that has been widely used to assess $p27^{Kip1}$ expression in various malignant diseases, including breast cancer. Moreover, antibodies from different sources and comparison of immunohistochemistry results with Western blot gave similar results.[14] In the majority of reports, $p27^{Kip1}$ levels have been classified as low (nuclear $p27^{Kip1}$ staining in $<50\%$ tumor cells) or high (nuclear $p27^{Kip1}$ staining in $\geq 50\%$ tumor cells).[13-19] Therefore, we selected this cutoff in the present study. Nevertheless, we obtained comparable results when $p27^{Kip1}$ expression was analyzed as a continuous variable (data not shown). However, there is clearly a need to standardize the $p27^{Kip1}$ detection assays and scoring systems to ensure that determination of $p27^{Kip1}$ levels is comparable between laboratories before $p27^{Kip1}$ can become part of the routine processing of pathologic tumor specimens and used as a new predictive marker for specific treatments.

The treatment protocols of the present study represent reasonable treatment options with regard to the management of premenopausal women with early-stage hormone receptor–positive breast cancer. Various randomized trials have shown that ovarian ablation with or without tamoxifen and standard chemotherapy regimens like CMF have similar benefits for premenopausal women with early-stage receptor-positive breast cancer.[31] Thus, both the panelists of the 2001 Consensus Meeting in St Gallen and the National Institutes of Health Consensus Development Panel have suggested that ovarian ablation is a reasonable adjuvant treatment option for those patients.[32,33] Moreover, the panelists at St. Gallen concluded that combined endocrine therapy may be regarded as a proper treatment option for premenopausal women with endocrine-responsive disease.[32] Our present results may help to identify more precisely those patients who would benefit most from combined endocrine therapy.

In conclusion, our results suggest that $p27^{Kip1}$ may be a useful marker for the selection of patients for adjuvant combination endocrine therapy, but this requires further confirmation by prospective studies before clinical implementation.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

APPENDIX

The appendix is included in the full-text version of this article, available on-line at www.jco.org. It is not included in the PDF (via Adobe® Acrobat Reader®) version.

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