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p27 and Skp2 immunoreactivity and its clinical significance with endocrine and chemo-endocrine treatments in node-negative early breast cancer

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Background: Low p27 and high Skp2 immunoreactivity are associated with a poor prognosis and other poor prognostic features including resistant phenotypes and antiestrogen drug resistance. We investigated these proteins in two International Breast Cancer Study Group trials studying node-negative early breast cancer.

Patients and methods: Trial VIII compared chemotherapy followed by goserelin with either modality alone in premenopausal patients. Trial IX compared chemotherapy followed by tamoxifen with tamoxifen alone in postmenopausal patients. Central Pathology Office assessed p27 and Skp2 expression in the primary tumor by immunohistochemistry among 1631 (60%) trial patients.

Results: p27 and Skp2 were inversely related; 13% of tumors expressed low p27 and high Skp2. Low p27 and high Skp2 were associated with unfavorable prognostic factors including larger size and higher grade tumors, absence of estrogen receptor and progesterone receptor, human epidermal growth factor receptor 2 overexpression and high Ki-67 (each $P < 0.05$). Low p27 and high Skp2 were not associated with disease-free survival ($P = 0.42$ and $P = 0.48$, respectively). The relative effects of chemo-endocrine versus endocrine therapy were similar regardless of p27 or Skp2.

Conclusions: We confirm the association of low p27 and high Skp2 with other poor prognostic features, but found no predictive or prognostic value, and therefore do not recommend routine determination of p27 and Skp2 for node-negative breast cancer.

Key words: breast cancer, chemotherapy, hormonal therapy, p27, Skp2

introduction

The prognosis of patients with early breast cancer is primarily determined by traditional tumor factors such as tumor stage, size, extent of nodal spread and the presence or absence of metastases [1]. The biological markers estrogen receptor (ER), progesterone receptor (PgR) and human epidermal growth factor receptor 2 (HER2) have been widely accepted for routine use, serving also as predictors of therapy responsiveness [2]. p27 is a member of the Cip/Kip family of cyclin-dependent kinase inhibitors, expressed at a high level in G_0 cells, which promotes cell cycle arrest and apoptosis. The S-phase kinase-associated proteins Skp2 are required for the ubiquitination and consequent degradation of p27 which allows cells to enter the S-phase.

In breast tumors, low p27 and high Skp2 are more frequently associated with ER-negative phenotype, high histological grade and poor prognosis. Traub et al. [3] described low p27/high Skp2 as a poor prognostic factor and identified a subset with Skp2 expressed at low levels despite low p27, perhaps due to low levels but hyperactive Skp2 or other molecular mechanisms [4]. In early-stage breast carcinoma, a decreased immunoreactivity for p27 was correlated with HER2 overexpression and with benefit from one course of perioperative chemotherapy [5]. Furthermore,

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a deregulated expression of Skp2 in breast cancer cells might have a relevant role in the development of resistance to antiestrogen drugs, whereas a normal p27 expression could be considered an independent predictor of responsiveness to hormonal therapy [6]. Other papers report a higher Skp2 expression in a subset of ER-negative aggressive breast carcinomas [7] and the development of trastuzumab resistance in association with loss of p27 nuclear expression in breast cancer cells [8].

The present study was designed to assess whether p27 and Skp2, alone or together, could be useful to identify subgroups of patients more likely to benefit from adjuvant endocrine or chemo-endocrine treatments and to analyze their prognostic and predictive value in comparison with previously reported data. For this purpose, we have chosen patients enrolled in two randomized clinical trials comparing adjuvant endocrine therapy and/or chemotherapy in node-negative invasive breast cancer.

patients and methods

International Breast Cancer Study Group trials VIII and IX

International Breast Cancer Study Group (IBCSG) trials VIII and IX [9, 10] were randomized clinical trials comparing adjuvant endocrine therapy alone and sequential chemotherapy followed by endocrine therapy for node-negative invasive breast cancer among premenopausal (trial VIII) and postmenopausal (trial IX) patients. In both trials, patients with ER-positive, ER-negative and ER-unknown tumors (ER-unknown status allowed only if ER determination was not possible because of lack of tumor material) were eligible until 1998; at that time, protocol amendments restricted enrollment to patients with ER-positive tumors. Over 94% of patients were randomized before the amendments' release. Institutional review boards reviewed and approved the protocols, and an informed consent was required according to the criteria established within the individual countries.

treatment regimens

Trial VIII evaluated whether sequential treatment with six 28-day courses of 'classical' cyclophosphamide, methotrexate, fluorouracil (CMF) chemotherapy followed by 18 monthly s.c. implants of goserelin significantly improved disease-free survival (DFS) as compared with either six 28-day courses of classical CMF alone or 24 monthly implants of goserelin alone. From 1990 through 1999, a total of 1063 assessable pre/perimenopausal patients with node-negative disease were randomized [9]. Trial IX evaluated whether sequential treatment with three 28-day courses of classical CMF chemotherapy followed by tamoxifen for 57 months significantly improved DFS as compared with tamoxifen alone for 5 years. From 1988 through 1999, a total of 1669 eligible and assessable postmenopausal patients with node-negative disease were randomized [10].

pathology cohort

Retrospective tissue collection was carried out for patients randomized in IBCSG trials VIII and IX in accordance with institutional guidelines and national laws. Archival tumor material (blocks and/or slides) was available and assessable by the IBCSG Central Pathology Laboratory among 953 of 1063 (90%) trial VIII patients and 1541 of 1669 (91%) trial IX patients. About 30% of patients had only four unstained slides available which were used for ER, PgR, HER2 and Ki-67 labeling index (LI)—and their tumors could not be assessed for p27 and Skp2 expression. Thus, material was available to assess p27 and Skp2 among 647 (61%) of 1063 trial VIII patients and 984 (59%) of 1669 trial IX patients.

immunohistochemistry

In the IBCSG Central Pathology Laboratory in Milan, Italy, expression of ER, PgR, HER2, Ki-67, p27 and Skp2 in the primary tumors was determined by immunohistochemistry (IHC) as previously described [11] without knowledge of treatment assignment or outcome. The tumor sections were incubated with the specific primary monoclonal antibodies to ER (Dako, Glostrup, Denmark, clone 1D5, 1 : 100 dilution), PgR (Dako, clone 1A6, 1 : 800 dilution), Ki-67 (Dako, clone Mib-1, 1 : 200 dilution), p27 (Transduction Laboratories, Lexington, KY, 1 : 600 dilution) and Skp2 (Zymed, San Francisco, CA, clone: 1G12E9, 1 : 10 dilution). HER2/neu immunoreactivity was evaluated using the HercepTest kit (Dako), as recommended by the manufacturer, and the tumors were scored for the intensity of immunostaining, the completeness of cell membrane staining and the percentage of immunoreactive neoplastic cells by using a four-tier scale (from 0 to 3+), as recommended by the Food and Drug Administration [12].

statistical methods

ER and PgR status were classified by dichotomizing IHC expression as present (≥1% immunoreactive cells) or absent (0%). HER2 was considered as overexpressed if the intensity was scored 3+. Ki-67 LI was classified as high for ≥19% immunoreactive cells which was the median value of distribution [13]. Tumor p27 expression was considered as low for <50% and normal for ≥50% immunoreactive cells [5, 14] and Skp2 expression was considered as high for ≥10% immunoreactive cells [4], which corresponded closely to a cut point of >5% immunoreactive cells [3] because few tumors were scored in the range of 6%–9% immunostaining.

Logistic regression modeling was used to examine the association of tumor grade and size, ER, PgR, HER2 and Ki-67 LI expression and the interactions of these variables with both p27 and Skp2 status, controlling for menopausal status.

DFS was defined as in the respective trial manuscripts as the length of time from the date of randomization to any relapse including ipsilateral breast recurrence or the appearance of a second primary malignancy (including contralateral breast cancer), or death, whichever occurred first. Hazard ratios (HRs), two-sided 95% confidence intervals (CIs) and statistical tests were estimated from Cox proportional hazards models. The nonparametric subpopulation treatment effect pattern plot (STEPP) methodology [15] was used to investigate trends in treatment effect differences across the continuum of p27 expression; a meaningful STEPP analysis could not be undertaken for Skp2 because of the highly skewed distribution. The analysis used SAS version 9.1 (SAS Institute Inc., Cary, NC) and S-PLUS version 6.1 (Insightful Corp., Seattle, WA).

results

A total of 647 (61%) of 1063 premenopausal patients from IBCSG trial VIII and 984 (59%) of 1669 postmenopausal patients from IBCSG trial IX had tumor material available and assessable for p27 and/or Skp2 immunoreactivity and were included in the analysis. Median age at randomization was 45 years [interquartile range (IQR) 41–48 years] and 61 years (IQR 56–65 years) among premenopausal and postmenopausal patients, respectively. In total, 705 (43%) patients had been randomized to receive endocrine therapy alone (198 goserelin and 507 tamoxifen) and 703 (43%) to chemo-endocrine therapy (226 CMF→goserelin; 477 CMF→tamoxifen). Median duration of follow-up was 10 years in the analysis cohort (9.4 and 10.3 years in the analysis cohorts from trials VIII and IX, respectively). The analysis cohorts are representative of the

overall trial cohorts (data not shown), with the exception of an underrepresentation of patients with smaller and lower grade tumors in the analysis cohort.

p27 and Skp2 immunoreactivity

The median level of tumor p27 expression was 44% (IQR 15%–76%; range, 0%–98%) immunoreactive cells and 55% (875 of 1598) of patients' tumors were classified as having low p27 expression (i.e. <50% immunoreactive cells). Among premenopausal patients, the median p27 expression was 42% (IQR 16%–74%) and among postmenopausal patients in trial IX, the median expression was 46% (IQR 15%–77%). In total, 56% and 54% of pre- and postmenopausal patients' tumors were classified as having low p27 expression, respectively, and thus low p27 expression was independent of menopausal status ($P = 0.28$, Table 1).

Table 1. Patient menopausal status and tumor features according to tumor p27 and Skp2 expression status

	p27			Skp2		
	Low (<50%)	Normal (≥50%)	*P* value	Normal (<10%)	High (≥10%)	*P* value
Patients, *n* (%)	875 (55)	723 (45)		1274 (80)	315 (20)	
Menopausal status			0.28			<0.001
Premenopausal	357 (56)	275 (44)		480 (75)	157 (25)	
Postmenopausal	518 (54)	448 (46)		794 (83)	158 (17)	
ER			<0.001			<0.001
Absent (0%)	261 (83)	54 (17)		168 (53)	150 (47)	
Present (≥1%)	603 (48)	664 (52)		1093 (87)	162 (13)	
Median ER% (IQR)	71% (0–90)	90% (75–95)		90% (62–95)	2% (0–88)	
PgR			<0.001			<0.001
Absent (0%)	367 (74)	132 (26)		322 (64)	179 (36)	
Present (≥1%)	498 (46)	587 (54)		941 (88)	134 (12)	
Median PgR% (IQR)	10% (0–76)	60% (5–90)		45% (0–90)	0% (0–60)	
ER/PgR status			<0.001			<0.001
ER−/PgR−	251 (83)	51 (17)		161 (53)	144 (47)	
ER−/PgR+	10 (77)	3 (23)		7 (58)	5 (42)	
ER+/PgR−	114 (58)	81 (42)		159 (82)	34 (18)	
ER+/PgR+	485 (46)	580 (54)		928 (88)	128 (12)	
HER2			0.006			0.02
Overexpressed (3+)	157 (63)	93 (37)		186 (74)	64 (26)	
Not (0, 1+, 2+)	713 (53)	625 (47)		1080 (81)	251 (19)	
ER/PgR/HER2 status			<0.001			<0.001
ER−/PgR−/HER2−	178 (82)	39 (18)		108 (50)	110 (50)	
Other	691 (50)	683 (50)		1160 (85)	204 (15)	
Ki-67 LI			<0.001			<0.001
Normal (<19%)	318 (47)	352 (53)		628 (94)	40 (6)	
High (≥19%)	458 (58)	334 (42)		548 (69)	244 (31)	
Median KI-67 LI% (IQR)	22% (13–39)	18% (11–28)		17% (11–27)	43% (26–66)	
Tumor grade			<0.001			<0.001
1	89 (37)	152 (63)		224 (95)	13 (5)	
2	346 (50)	342 (50)		598 (88)	83 (12)	
3	407 (66)	211 (34)		403 (65)	216 (35)	
Tumor size (cm)			0.04			<0.001
≤2	488 (53)	441 (47)		784 (85)	138 (15)	
>2	368 (58)	267 (42)		455 (72)	177 (28)	
Skp2			<0.001			–
Normal (<10%)	643 (52)	600 (48)		–	–	
High (≥10%)	208 (66)	105 (34)		–	–	
Unknown	24	18		–	–	
Median Skp2% (IQR)	0% (0–5)	0% (0–2)		–	–	
p27						<0.001
Normal (≥50%)	–	–		600 (85)	105 (15)	
Low (<50%)	–	–		643 (76)	208 (24)	
Unknown	–	–		31	2	
Median p27% (IQR)	–	–		47% (16–80)	36% (13–60)	

Percentages sum across the rows; there are patients with unknown values of the different tumor features.
ER, estrogen receptor; IQR, interquartile range; PgR, progesterone receptor; HER2, human epidermal growth factor receptor 2; LI, labeling index.

The median level of tumor Skp2 expression was 0% (IQR 0%–5%; range 0%–95%) immunoreactive cells, indeed only 43% (686 of 1589) showed any reactive cells, and 20% (315 of 1589) of patients' tumors were classified as having high Skp2 expression (i.e. ≥10% immunoreactive cells). Among premenopausal patients, the median level was 0.5% (IQR 0%–5%) and among postmenopausal patients was 0% (IQR 0%–3%). In total, 25% and 17% of pre- and postmenopausal patients' tumors were classified as having high Skp2 expression, respectively, which was significantly different ($P < 0.001$).

There was an inverse relationship between p27 and Skp2 immunoreactivity; 66% of tumors expressing high Skp2 also had low p27 immunoreactivity as compared with 52% of tumors expressing normal Skp2 levels ($P < 0.001$). This relationship was independent of menopausal status: among premenopausal patients, 66% versus 53% of tumors expressing high versus normal Skp2 also had low p27 immunoreactivity, and among postmenopausal patients 66% versus 51% of tumors having high versus normal Skp2 expression also had low p27 immunoreactivity. Overall, 13% (208/1556 with both p27 and Skp2 measured) of tumors expressed high Skp2 and low p27.

In univariate analyses, low p27 immunoreactivity was associated with higher tumor grade, larger tumor size, high Ki-67 LI, ER-absent, PgR-absent and HER2 overexpressing tumors (each $P < 0.05$, Table 1). In total, 82% of tumors that did not express ER or PgR and did not overexpress HER2 also had low p27 immunoreactivity, thus it appears that low p27 expression is commonly found in association with the so-called triple-negative phenotype. In multivariable logistic regression analyses controlling for menopausal status, the ER and PgR status of the tumor and tumor grade remained statistically significant in the final model (each $P < 0.01$). Interactions between the variables were explored but none were found to be statistically significant.

Similarly, in univariate analyses, high Skp2 immunoreactivity was associated with higher tumor grade, larger tumor size, high Ki-67 LI, ER-absent, PgR-absent and HER2 overexpressing tumors (each $P < 0.05$, Table 1). In multivariable logistic regression analyses controlling for menopausal status, the ER and Ki-67 LI status of the tumor and the tumor grade and size remained statistically significant in the final model (each $P < 0.01$). Interactions between the variables were explored but none were found to be statistically significant.

Finally, to the multivariable logistic regression model for p27 expression status (which included menopausal status, ER, PgR and grade), Skp2 expression status was added and interactions of Skp2 with other variables were explored—in particular with ER status and HER2 status. When Skp2 expression status was added to the model, it was not associated with p27 immunoreactivity ($P = 0.97$). There was a suggestion of an interaction of Skp2 with ER status ($P = 0.08$), which would indicate that the association of Skp2 with p27 status differs depending on whether or not the tumor expresses ER: among tumors with ER present, low p27 immunoreactivity was observed more often in high than in normal Skp2-expressing tumors (54% versus 47% unadjusted), whereas among tumors absent of ER, low p27 was observed less often in high than in normal Skp2-expressing tumors (80% versus 86% unadjusted) (Figure 1). When the interaction with HER2 status was also considered, there was no evidence that HER2 status influenced the association of p27 and Skp2 immunoreactivity.

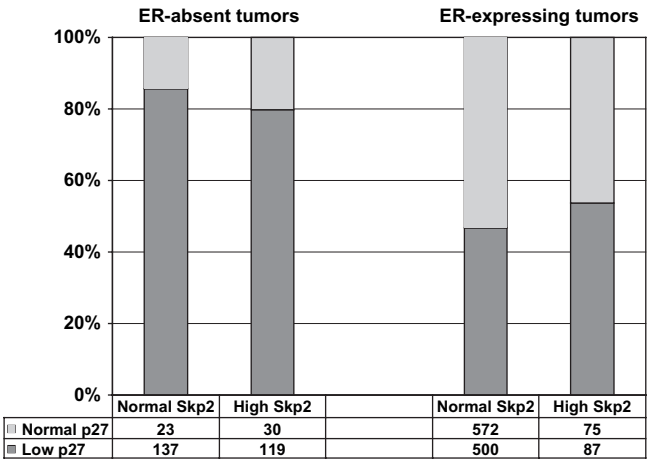

Figure 1. Frequency of low p27 immunoreactivity according to Skp2 and estrogen receptor status of the tumor. Tumor p27 expression was considered as low for <50% and as normal for ≥50% immunoreactive cells; Skp2 expression was considered as high for ≥10% and as normal for <10% immunoreactive cells.

patient outcome for endocrine-responsive tumors

Low p27 immunoreactivity was not associated with DFS (HR = 1.15, 95% CI = 0.93–1.41, $P = 0.21$) nor was high Skp2 expression associated with DFS (HR = 1.30, 95% CI = 0.98–1.74, $P = 0.07$) in univariate analyses among patients with ER-expressing tumors (Figure 2). There was no evidence of interaction of p27 and Skp2 status with DFS ($P = 0.55$) (Figure 2C).

Because of the associations of other tumor features with p27 and Skp2 expression, we examined whether there was heterogeneity in their relationship with DFS according to subgroups defined by other tumor features (i.e. interactions). There was no other tumor feature for which the association of p27 status with DFS clearly varied in subgroups from the overall effect (data not shown). There was also no evidence of an interaction of p27 status with randomized treatment regimen ($P = 0.42$), indicating that the treatment effect was not different among patients with tumors expressing low p27 (HR = 0.99, 95% CI = 0.73–1.35 comparing chemo-endocrine versus endocrine therapy alone) and those with normal p27 (HR = 0.84, 95% CI = 0.61–1.16). This is further illustrated in the STEPP analysis of 10-year DFS according to quantitative degree of p27 immunoreactivity of the tumor (Figure 3). There is no clear pattern of benefit for one treatment as compared with the other across p27 levels, nor is there a clear pattern to indicate that endocrine therapy alone might be less effective at lower levels of p27 expression as the curve remains near 69% of 10-year DFS for all degrees of p27 immunoreactivity.

Similarly for Skp2, there was no other tumor feature for which the association of Skp2 status with DFS clearly varied in subgroups from the overall association. There was no

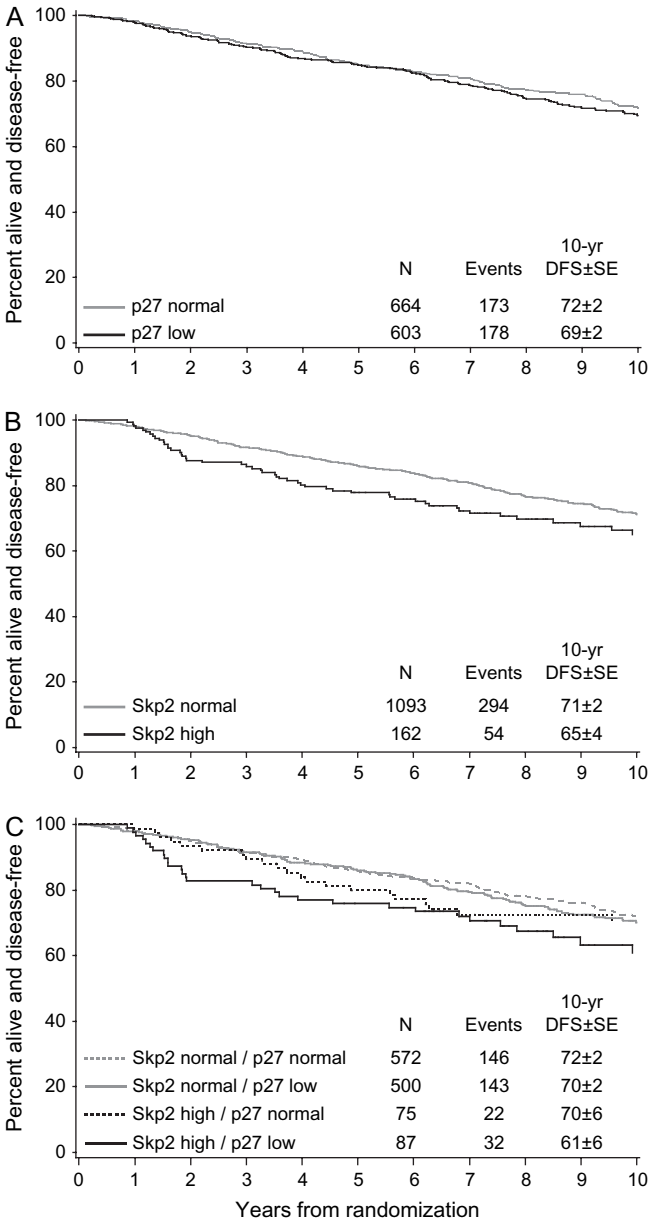

Figure 2. Disease-free survival among patients with estrogen receptor-expressing tumors, by (**A**) tumor p27 status; (**B**) Skp2 status and (**C**) both p27 and Skp2 status. Tumor p27 expression was considered as low for <50% and as normal for ≥50% immunoreactive cells; Skp2 expression was considered as high for ≥10% and as normal for <10% immunoreactive cells.

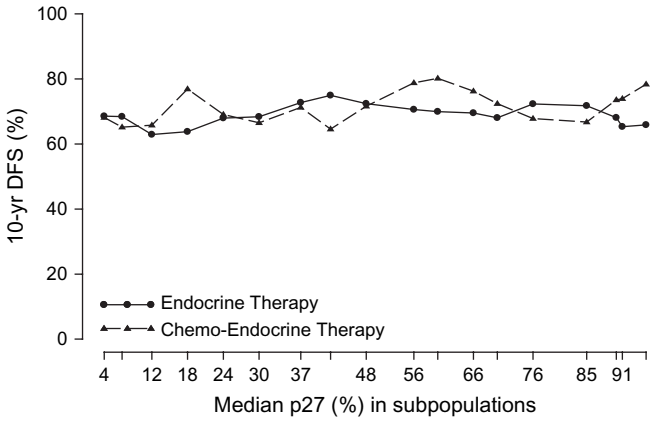

Figure 3. Subpopulation treatment effect pattern plot (STEPP) analysis of disease-free survival (DFS) among patients with estrogen receptor-expressing tumors, according to p27 immunoreactivity of the tumor. The analysis uses a sliding-window approach to define several overlapping subpopulations of patients on the basis of the level of p27 immunoreactivity. The *x*-axis indicates the median value of p27 for patients and the *y*-axis indicates the 10-year DFS for patients in each subpopulation. Each subpopulation contains ∼170 patients and slides by ∼30 patients.

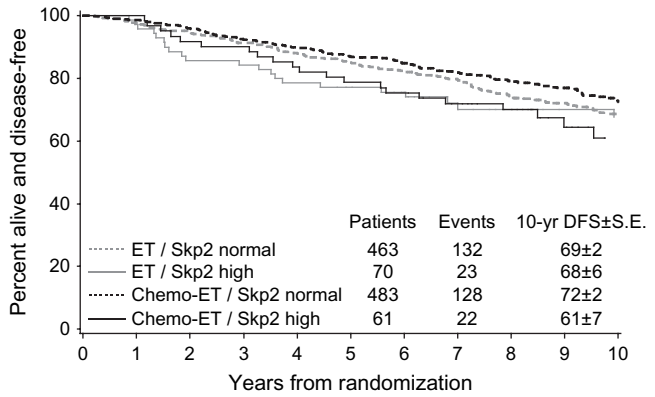

Figure 4. Disease-free survival among patients with estrogen receptor-expressing tumors, by tumor Skp2 status and randomized treatment regimen. Skp2 expression was considered as high for ≥10% and normal for <10% immunoreactive cells.

interaction with treatment regimen ($P = 0.48$), indicating that the treatment effect was not different among patients with tumors expressing high Skp2 (HR = 1.07, 95% CI = 0.60–1.93) and those with normal Skp2 (HR = 0.88, 95% CI = 0.69–1.13) (Figure 4).

patient outcome for endocrine-nonresponsive tumors

The univariate associations of menopausal status, randomized treatment regimen and tumor factors with DFS among the 324 patients with tumors absent of ER expression were also studied. No variable, other than treatment regimen, was statistically significantly associated with DFS, including p27 status ($P = 0.70$) and Skp2 status ($P = 0.30$). A STEPP analysis of 10-year DFS according to p27 expression of the tumor shows consistent benefit of chemo-endocrine versus endocrine therapy alone, regardless of p27 level (Figure 5). There was a suggestion of a greater benefit of chemo-endocrine therapy at the very lowest levels of p27 expression, both because of a slightly better outcome on chemo-endocrine therapy and because of slightly poorer outcome on endocrine therapy alone, among the subpopulations of patients with tumors having the lowest degree of p27 immunoreactivity. Thus, if we consider quantitative degrees of p27 expression, there is some evidence that among patients with tumors

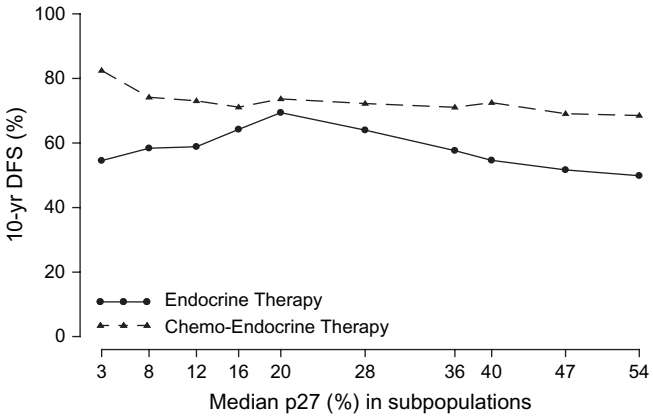

Figure 5. Subpopulation treatment effect pattern plot (STEPP) analysis of 10-year disease-free survival (DFS) among patients with tumors absent of estrogen receptors, according to p27 immunoreactivity of the tumor. The analysis uses a sliding-window approach to define several overlapping subpopulations of patients on the basis of the level of p27 immunoreactivity. The *x*-axis indicates the median value of p27 for patients and the *y*-axis indicates the 10-year DFS for patients in each subpopulation. Each subpopulation contains ~110 patients and slides by ~10 patients

absent of ER expression, the benefit of chemo-endocrine versus endocrine alone may be modified by p27 expression; we did not see this in patients with ER-expressing tumors.

discussion

Many studies, mainly on tumor material obtained from unselected populations of patients treated outside randomized clinical trials or with limited clinical follow-up, have indicated a prognostic or predictive value of low p27 expression, high Skp2 expression or both in patients with early breast cancer [3, 16–19] and other tumor types [20–24]. Our group has previously shown that low p27 expression was not associated with prognosis in a cohort of node-positive and node-negative early breast cancer patients treated with CMF-based chemotherapy [5]; in addition, these data indicated an inverse relationship between low p27 immunoreactivity and HER2 overexpression and a benefit of perioperative CMF chemotherapy in patients with tumors showing low p27 levels.

The findings of the current study confirm in a larger population that low p27 does not have an independent prognostic role in early breast cancer patients with node-negative disease, and show that high expression of Skp2 is also not associated with prognosis. These results, however, shed more light on the relationship between these two markers involved in cell cycle regulation and their relationship to other routinely used markers of treatment responsiveness, in particular hormone receptor status and HER2 status.

In the evaluation of patients of IBCSG trials VIII and IX, an inverse relationship between p27 and Skp2 was found: 66% of tumors expressing high Skp2 also had also low p27. Overall, 13% of tumors expressed high Skp2 and low p27. We did not observe relationships between antiestrogen actions and p27/Skp2 values, meaning that the biological activation/inactivation pathways are more complex.

A recent paper [3], analyzing 338 patients of which about half had node-positive and half node-negative disease and 71% had ER-positive tumors, found 34% of these patients with tumors having both high Skp2 and low p27. The authors observed that this group had a significantly worse DFS including the subgroup with node-negative disease. On the other hand, in the univariate analysis normal p27 was not associated with worse DFS in this subgroup. It is, however, important to highlight that this cohort of patients was not analyzed within ER-positive and ER-negative cohorts and included patients with node-negative and node-positive disease treated heterogeneously. In our cohort of patients, 13% had low p27/high Skp2 and all had node-negative disease, we observed no association between normal/low p27 and DFS or between high/normal Skp2 and DFS among patients with endocrine-responsive tumors.

Several authors have indicated that p27 and/or Skp2 have some value in predicting response to breast cancer treatments [14, 15, 19, 25, 26]. In our study, considering patients with endocrine-responsive and nonresponsive tumors separately, relative effects of chemo-endocrine versus endocrine therapy on DFS were similar regardless of p27 or Skp2 status. The findings indicated a larger benefit for the subgroup of patients with ER-absent tumors with particularly low levels of p27 receiving CMF chemotherapy before endocrine therapy as compared with endocrine therapy alone. These results are consistent with our previous study [5]. However, on the basis of the data presented in this report we cannot recommend routine determination of p27 and Skp2 outside clinical trials in any population of patients, particularly as predictor of treatment responsiveness.

The molecular machinery regulating cell cycle and in particular the entry into cell cycle and the transition from G_0 to G_1 is known to be complex and not restricted to the two molecules studied here, despite their key role. The data of Radke et al. [27] support a model where different pathways, some Skp2 dependent and some Skp2 independent, may converge and lead to p27 inactivation. Moreover, recent research has shown an association of breast tumors with the so-called triple-negative phenotype (ER/PgR/HER2-negative) with unfavorable prognosis[28]. The common down-regulation of p27 in these tumor types found in our study indicates that strategies interfering with p27 inactivation could be potentially useful in this particular tumor type, where innovative treatments are needed. In addition, p27 inactivation during the development of resistance to anti-HER2 treatment (e.g. trastuzumab) should be studied in the future context of this targeted therapy.

Our results confirm an inverse relationship between p27 and Skp2 expression which was independent of menopausal status and the association of low p27 and high Skp2 with other unfavorable prognostic features of the tumor. We observed a possible interaction between Skp2 and ER status in their relationship with p27 status, but this interaction was not statistically significant. This relationship should be further investigated, and emphasizes the need to carefully consider ER status of the tumors when studying the

relationship of p27 and Skp2. We did not observe a prognostic or predictive role of p27 or Skp2. DFS and the relative effects of chemo-endocrine versus endocrine therapy alone as used in these trials were not influenced by p27 and Skp2 immunoreactivity.

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appendix 1 International Breast Cancer Study Group

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