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Genomic grade adds prognostic value in invasive lobular carcinoma†

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Background: The prognostic value of histologic grade (HG) in invasive lobular carcinoma (ILC) remains uncertain, and most ILC tumors are graded as HG2. Genomic grade (GG) is a 97-gene signature that improves the prognostic value of HG. This study evaluates whether GG may overcome the limitations of HG in ILC.

Methods: Gene expression data were generated from frozen tumor samples, and GG calculated according to the expression of 97 genes. The prognostic value of GG was assessed in a stratified Cox regression model for invasive disease-free survival (IDFS) and overall survival (OS).

Results: A total of 166 patients were classified by GG. HG classified 33 (20%) tumors as HG1, 120 (73%) as HG2 and 12 (7%) as HG3. GG classified 106 (64%) tumors as GG low (GG1), 29 (17%) as GG high (GG3) and 31 (19%) as equivocal (cases not classified as GG1 or GG3). The median follow-up time was 6.5 years. In multivariate analyses, GG was associated with IDFS [$HR_{GG3\ vs\ GG1}$ 5.6 (2.1–15.3); $P < 0.001$] and OS [$HR_{GG3\ vs\ GG1}$ 7.2, 95% CI (1.6–32.2); $P = 0.01$].

Conclusions: GG outperformed HG in ILC and added prognostic value to classic clinicopathologic variables, including nodal status.

Key words: breast cancer, genomics, lobular carcinoma, prognosis

introduction

Invasive lobular carcinoma (ILC) is the second most common breast cancer subtype, accounting for 10% to 15% of breast cancers. ILC differs from invasive ductal carcinoma (IDC) with respect to epidemiology, clinical presentation and responsiveness to systemic treatments [1–3].

The median incidence of ILC is disproportionately high when compared with IDCs, particularly in Western countries and among women older than 50 [4]. Moreover, ILCs are less responsive to chemotherapy, more frequently bilateral at the initial diagnosis and more prone to present bone, gastrointestinal, peritoneal and ovarian metastases than IDCs [2].

ILCs are often hormone receptor-positive tumors, HER2-negative and present low-to-intermediate histologic grade (HG 1 or 2). Nevertheless, a variant form of ILC named pleomorphic invasive lobular carcinoma (PILC) is characterized by a higher grade (HG3) than classical ILC and often classified as HER2-positive [5].

In contrast to IDC, the prognostic value of HG in ILC is not well established. Standard HG assessment by the Elston–Ellis method is based on the semi-quantitative evaluation of three morphologic features (tubule formation, nuclear pleomorphism and mitotic count) [6]. However, ILC tumors lack tubule formation, which hinders the process of grading in this histologic subtype and results in the classification of approximately two-thirds of ILC as HG2, which is non-informative for treatment decision making.

Over the last decade, a number of genomic signatures have shed light onto breast cancer classification, allowing breast cancer care to become more personalized [7]. With respect to the management of estrogen receptor (ER)-positive breast cancer, gene expression signatures have demonstrated their ability to provide prognostic and predictive information beyond what is possible with current classical clinicopathologic parameters alone [7], though their value for use in clinical

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practice is still under investigation in prospective clinical trials [8, 9].

Nevertheless, most studies using genomic signatures have not considered different histologic subtypes separately. The genomic grade (GG) is a 97-gene signature that improves the prognostic value of HG in IDC [10]. With a view towards improving treatment decision making for patients with ILC in the future, we evaluated the prognostic performance of GG in tumors from 166 patients diagnosed with primary ILC in this present study.

patients and methods

patient selection

A retrospective series of frozen tumor samples from patients treated in three different centers in Belgium and France [Institut Jules Bordet (IJB), Brussels; Institut Paoli Calmettes, Marseille; and Cliniques Universitaires Saint Luc, Brussels] was selected from the centers' tissue banks according to the following criteria: the tumor was primary ILC (classical ILC or PILC histotype) diagnosed between January 1990 and December 2005; axillary lymph node-negative or node-positive; no age limit; minimum 5 years' of clinical follow-up information was available; and there were no previous malignancies, except non-melanoma skin cancer. All patients were treated by breast surgery, followed by chemotherapy, endocrine therapy or radiotherapy as indicated and per local guidelines. The study protocol was approved by the ethics committee at each participating center.

tumor samples

Tumor samples classified either as classical ILC or PILC, with no other invasive histologic type, were included in this analysis. E-cadherin staining was performed at the time of diagnosis in cases of doubt about ILC versus IDC diagnosis. Tumor size, HG (Elston–Ellis method), lymph node status, Ki67 labeling index, ER and progesterone receptor (PR) were based on the assessment provided by the local breast cancer pathologists at the time of diagnosis. Ki67 was evaluated either as a continuous or categorical variable. A Ki67 cut point of 14% (low <14% and high ≥14%) was applied to this population, because this is the one considered best for separating luminal A from luminal B breast cancer molecular subtypes as defined by Perou et al. [11, 12]. Owing to variations in hormone receptors and HER2 assessment over time, we also evaluated ER and HER2 status using *ESR1* and *ERBB2* gene expression as recently published (supplementary Table S1, available at *Annals of Oncology* online) [13].

microarrays

Using Map*Quant* Path kits (Ipsogen), frozen tumor samples (10 × 20-μm-thick sections) immersed in RNAlater® and a representative hematoxylin and eosin (H&E) slide from eligible patients were sent from the three participating centers to a centralized laboratory (DNA Vision, Charleroi, Belgium) at room temperature. RNA extraction was performed on samples containing a minimum of 30% invasive tumor cells on the representative H&E slide. Cellularity was centrally evaluated at the IJB and subsequently at the Institut de Pathologie et de Génétique (Charleroi, Belgium). Microarray analyses were performed using Affymetrix U133 Plus 2.0 arrays at DNA Vision. GG was computed using the Ipsogen Map*Quant* Dx protocol (supplementary Table S2, available at *Annals of Oncology* online), which defines tumors as low GG (GG1) or high GG (GG3), based on GG values. To ensure robustness and accuracy of genomic grading as per the Map*Quant* protocol, when the GG value of a sample could not be ascribed with a probability ≥75% to either GG1 or GG3, it was defined as equivocal.

study end points

The grading classification performance of GG was evaluated in the overall population and in the subsets of classical and PILC. The associations between GG, invasive disease-free survival (IDFS) and overall survival (OS) were evaluated. IDFS was defined as the interval between the date of breast surgery and the date of any invasive breast recurrence (local, regional, contralateral breast, distant) or death [14]. Patients still alive at the last follow-up and with no evidence of any invasive breast disease were censored. OS was defined as time from breast surgery to death from any cause, with patients still alive at the last follow-up being censored.

statistical analysis

GG and HG classification were compared using cross-tabulation. IDFS and OS were estimated by Kaplan–Meier curves. The median follow-up was evaluated by the reverse Kaplan–Meier method.

The prognostic value of GG was evaluated by log-rank tests and multivariate Cox regression analyses, both stratified by center. Cox models included age (categories), pathologic tumor size (categories), number of histologically involved nodes (categories), HG (1, 2 or 3), HER2 status (negative or positive), ILC histotype (classical or PILC) and adjuvant systemic chemotherapy. The significance of individual variables in the Cox model was evaluated using Wald statistics. The proportional hazards assumptions were tested by including interactions with log(time) in the models. There was no global significant non-proportional hazards pattern. The added value of GG both as a continuous variable and using the three categories (GG1, equivocal and GG3) to classical clinicopathologic variables was assessed by the likelihood ratio test (Δx^2) in the Cox regression models. The concordance-index (*c*-index) was computed to evaluate the added discrimination of GG to the clinicopathologic variables included in the Cox regression models.

Three different models were evaluated: (i) clinicopathologic variables alone (tumor size, nodal status, HG, ILC histotype, adjuvant chemotherapy); (ii) clinicopathologic variables plus continuous genomic grade index (GGI); (iii) clinicopathologic variables plus categorical GG. The larger the *c*-index, the better the predictability of a survival model: a value of 0.5 refers to no predictive discrimination, and a value of 1 indicates perfect discrimination of patients with different outcomes [15]. Cubic splines with 3 degrees of freedom were fitted to GGI in order to visualize its continuous relationship with 5-year predicted IDFS.

results

study population

A total of 243 tumor samples were sent for analysis, of which 225 had sufficient invasive tumor cells for the analysis. GG was successfully obtained for 174 samples. After merging the genomic and clinical datasets, eight patients were excluded as a result of clinical characteristics (four with mixed tumor histology, two with metastatic disease and two with non-breast synchronous malignancies). The 166 assessable patients were diagnosed between 1994 and 2005, with the exception of five patients diagnosed between 2005 and 2008. The median follow-up time among the 166 patients was 6.5 years (95% CI 5.7–9.3 years). The clinicopathologic characteristics of included patients are shown in Table 1. A total of 94% (156) and 95% (157) of patients were characterized as ER-positive by local

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Table 1. Summary of demographics and clinical characteristics of patients in the study

Patients	
Center 1	117 (71%)
Center 2	12 (7%)
Center 3	37 (22%)
Age at diagnosis	
Median (min–max)	59 (35–89)
<50 years	30 (18%)
≥50 years	136 (82%)
Tumor size	
Median (min–max)	2.7 (0.9–17)
≤2 cm	57 (34%)
>2 cm	109 (66%)
Nodal status	
Negative	86 (52%)
1–3 positive	48 (29%)
≥4 positive	30 (18%)
NA	2 (1%)
ER/PR status	
ER+/PR+	109 (66%)
ER+/PR−	47 (28%)
ER−/PR−	6 (4%)
ER−/PR+	4 (2%)
HER2 status[a]	
Negative	157 (95%)
Positive	9 (5%)
ILC histotype	
Classic	144 (87%)
Pleomorphic	22 (13%)
Histologic grade	
HG1	33 (20%)
HG2	120 (72%)
HG3	12 (7%)
Unknown	1 (1%)
Ki67	
Median	10 (0–60)
<14%	76 (46%)
≥14%	56 (34%)
Unknown	34 (20%)
Adjuvant therapy	
No	3 (2%)
HRT	152 (92%)
CT	97 (58%)
Unknown	1 (2%)
Genomic grade	
GG1	106 (64%)
Equivocal	31 (19%)
GG3	29 (17%)

Adj, adjuvant; HRT, hormonotherapy; CT, chemotherapy; ILC, invasive lobular carcinoma.

[a]HER2 assigned according to *ERBB2* gene expression.

assessment and *ESR1* gene expression, respectively. The overall discordance rate between genomic and IHC ER assessment was only 4.8% (8 of the 166 tumors). In the overall population, 43 patients (26%) experienced an IDFS event, and 20 patients (12%) an OS event. IDFS events according to nodal status were distributed as follows: node-negative, 11 (13%); 1–3 positive nodes, 12 (25%); and ≥4 positive nodes, 19 (63%). One IDFS event occurred in a patient with unknown nodal status. In the classical ($n = 144$) and PILC ($n = 22$) histotypes, 36 (25%) and 7 (32%) patients, respectively, experienced an IDFS event.

histologic and GG classification

In this study, 33 (20%) patients were classified as HG1, 120 (73%) as HG2 and 12 (7%) as HG3, as detailed in Figure 1. GG classified 106 (64%) tumors as GG1, 29 (17%) as GG3 and 31 (19%) as equivocal. Within the HG2 subgroup representing 73% of the cohort, GG reclassified 78% of the cases ($n = 94$) into GG1 (81%) and GG3 (19%), respectively. The overall concordance between HG1/HG3 and GG1/GG3 was 90%. In the subset of PILC, 1 (5%), 15 (68%) and 6 (27%) were classified as HG1, HG2 and HG3, respectively. GG reclassified the subset of PILC into 6 GG1 (27%), 6 GG3 (27%) and 10 equivocal (46%). When treating GG as a continuous variable (GGI), the median value was −0.70 (min–max −0.30 to 2.0; interquartile range: −1.63 to 0.10).

survival analysis: GG versus HG

The Kaplan–Meier curves for IDFS and OS are illustrated in Figure 2. GG was significantly associated with IDFS [hazard ratio (HR); $HR_{GG3\ vs\ GG1} = 3.3$ (1.7–6.8), $P = 0.001$] when compared with HG [$HR_{HG3\ vs\ HG1} = 2.5$ (0.9–7.1), $P = 0.09$]. Five-year IDFS values were 4.0 years (95% CI 3.4–4.5) for GG3 tumors and 4.7 years (95% CI 4.6–4.9) for GG1 tumors. Similarly, GG yielded a statistically significant prognostic value for OS [$HR_{GG3\ vs\ GG1} = 3.1$ (1.0–9.3), $P = 0.04$], which was not observed for HG [$HR_{HG3\ vs\ HG1} = 2.7$ (0.5–13.5), $P = 0.23$]. Five-year OS values were 4.5 years (95% CI 4.2–4.9) for GG3 tumors and 4.9 years (95% CI 4.8–5.0) for GG1 tumors.

survival analysis: GG versus Ki67

Ki67 labeling index using the pre-defined 14% cut-off point was not significant for IDFS [$HR_{Ki67High\ vs\ Ki67Low} = 1.8$ (0.9–3.4), $P = 0.09$], nor for OS [$HR_{Ki67High\ vs\ Ki67Low} = 1.3$ (0.5–3.3), $P = 0.58$], as shown in supplementary Figure S1, available at *Annals of Oncology* online. Ki67 evaluated as a continuous variable was not associated with either IDFS (HR for a one-unit increase in Ki67 HR = 1.0, $P = 0.11$) or OS (HR = 1.0, $P = 0.81$). In contrast, a one-unit increase in continuous GGI values corresponded to a significant HR of 1.7 (1.2–2.3) ($P = 0.001$) for IDFS and 1.6 (1.1–2.6) ($P = 0.03$) for OS.

prognostic models using clinicopathologic and GG data

Multivariate Cox proportional hazard models were built to interrogate whether GG adds prognostic value to clinicopathologic variables. Tables 2 and 3 described the Cox proportional hazard models for IDFS and OS before and after the addition of GG.3

As expected, nodal status was a strong prognostic factor for both IDFS [HR ≥ 4 positive nodes versus node negative = 10.7 (4.1–28.1), $P < 0.0001$] and OS [HR ≥ 4 positive nodes versus

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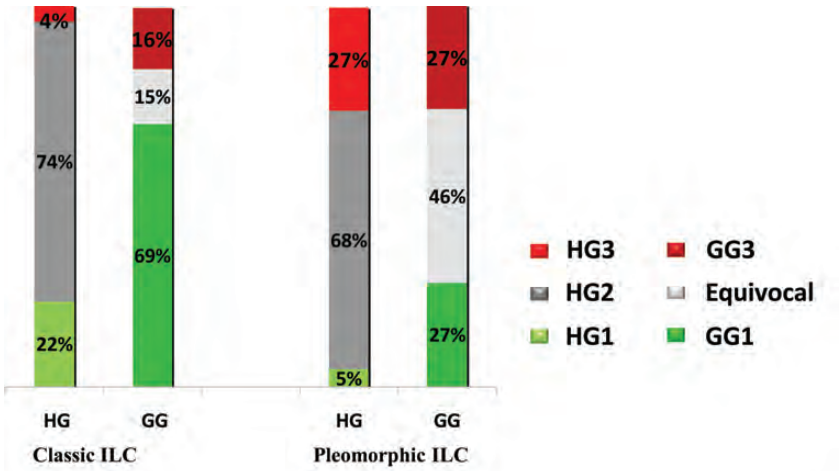

Overall	HG1	HG2	HG3	Total
GG1	28	76	1	105 (64%)
Equivocal	2	26	3	31 (19%)
GG3	3	18	8	29 (17%)
Total	33 (20%)	120 (73%)	12 (7%)	165

Figure 1. Distribution of histologic grade and genomic grade according to invasive lobular carcinoma histotype. GG, genomic grade; HG, histologic grade; ILC, invasive lobular carcinoma.

node negative = 36.9 (6.5–210.3), $P < 0.0001$]. Tumor size was a prognostic factor for IDFS [HR > 2 versus ≤2 cm = 3.6 (1.3–10.4), $P = 0.01$], but not for OS [HR > 2 versus ≤2 cm = 1.4 (0.3–6.4), $P = 0.68$]. HG (comparisons of HG2 versus HG1 or HG3 versus HG1) did not have prognostic value in the models either before or after the addition of GGI. Adjuvant chemotherapy was significantly associated with better prognosis in both models for IDFS [HR = 0.4 (0.2–0.9), $P = 0.02$] and OS [HR = 0.3 (0.1–0.9), $P = 0.04$]. The PILC histotype compared with classical ILC histotype was not significantly associated with worse prognosis in both models for IDFS [HR = 1.8 (0.7–4.5), $P = 0.24$] and OS [HR = 1.7 (0.3–8.4), $P = 0.51$]. HER2 status was not a prognostic factor for both IDFS and OS. In the model for IDFS, GG added prognostic value to clinicopathologic variables [$HR_{GG3\ vs\ GG1} = 5.6$ (2.1–15.3), Wald $P < 0.001$; $HR_{GG\ equiv\ vs\ GG1} = 2.8$ (1.2–6.8), Wald $P = 0.02$; $\Delta x^2 = 12.1$, likelihood ratio $P = 0.002$]. A worse OS was observed when GG was added to the evaluated clinicopathologic variables [$HR_{GG3\ vs\ GG1} = 7.2$, 95% CI (1.6 to 32.2), Wald $P = 0.01$; $HR_{GG\ equiv\ vs\ GG1} = 3.4$ (1.0–11.6), Wald $P = 0.005$; $\Delta x^2 = 12.1$; likelihood ratio $P = 0.002$]. The addition of GGI as a continuous variable to the same clinicopathologic variables yielded a significant HR for IDFS [$HR_{GG\ cont} = 2.1$ (1.4–3.2), Wald $P < 0.0001$; $\Delta x^2 = 13.5$, likelihood ratio $P < 0.0001$] and for OS [$HR_{GG\ cont} = 2.2$ (1.2–4.0), Wald $P = 0.01$; $\Delta x^2 = 6.4$, likelihood ratio $P = 0.01$].

The *c*-index showed increased predictive accuracy when GGI was added to clinicopathologic variables. The first model containing clinicopathologic variables yielded a *c*-index of 0.75 (95% CI 0.65–0.85), while the same model plus continuous or categorical GGI yielded c-indices of 0.79 (95% CI 0.71–0.87) and 0.81 (95% CI 0.75–0.88), respectively. The probability of a patient remaining free of an IDFS event at 5 years as a function of increasing GGI values is shown in Figure 3.

discussion

The presented results showed that GG added prognostic value to classic clinicopathologic variables in patients with ILC. Importantly, the lack of a subset of untreated patients with endocrine and/or chemotherapy and the possible interactions of adjuvant therapy on survival are limiting factors in the evaluation of the prognostic value of GG on patient's outcome.

Previous analyses of series of tumors have demonstrated a significant prognostic value for HG in ILC [16–18]. In the largest series [18], HG was evaluated across 517 cases of ILC and significantly associated with patient outcome. However, 34% of the included cases had non-classical histotypes of ILC (solid, alveolar, tubulobular and lobular mixed), which are often characterized as having either higher mitotic count or presence of tubule formation (the latter being absent in classical ILC). When the tubule formation component of HG was excluded from that analysis, there was no longer a statistically significant association between the remaining components (nuclear pleomorphism plus mitotic count) and prognosis.

Importantly, in a retrospective series including 931 ILC cases, Iorfida et al. [19] showed worse patient outcomes for non-classical ILC variants when compared with classical ILC cases. In the present series, 87% and 13% of tumors were classified as classical and PILC, respectively. In agreement with previous results, a trend towards worse outcome was observed among PILC patients.

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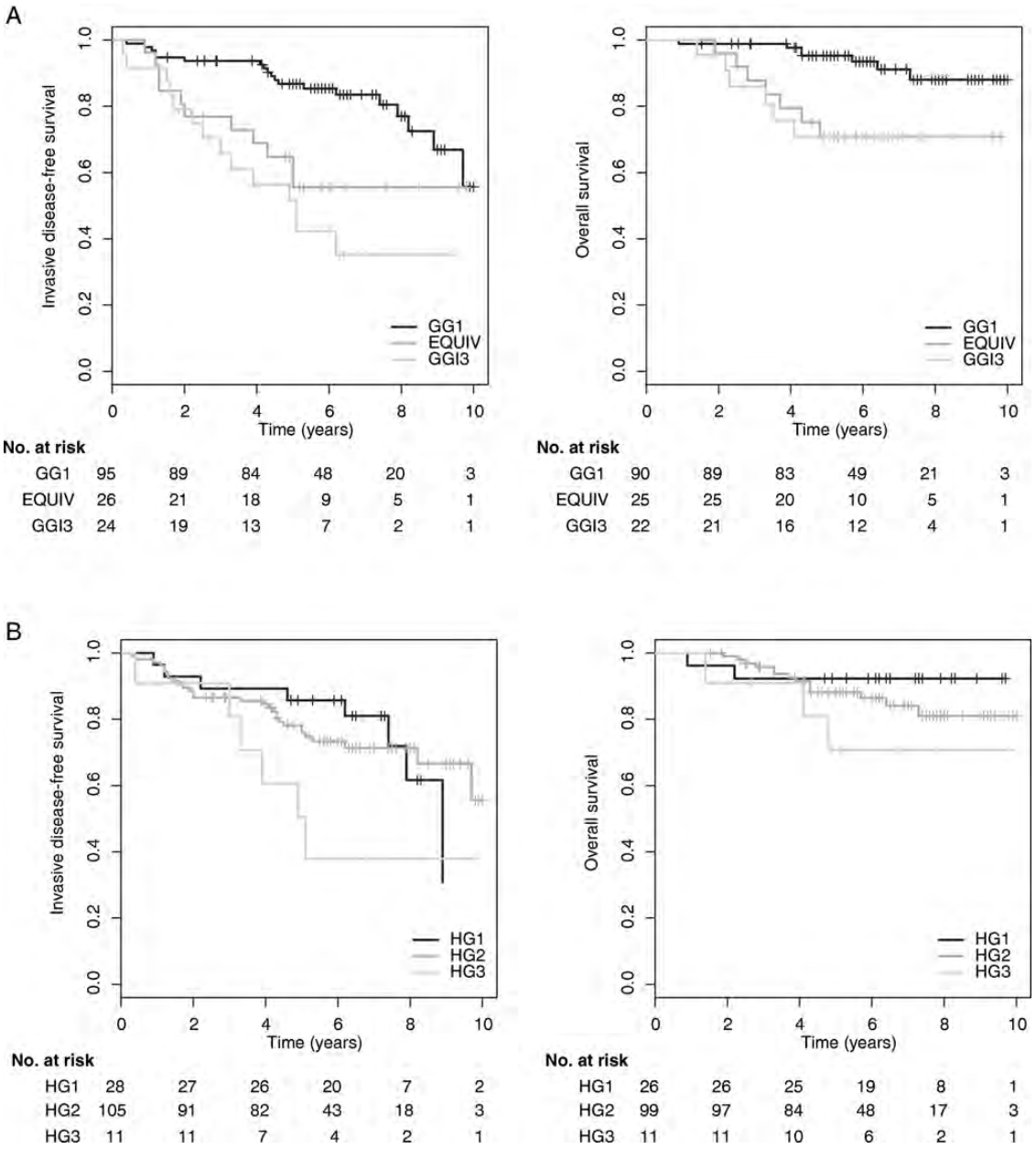

Figure 2. Kaplan–Meier plots of invasive disease-free survival and overall survival by genomic grade (A) and histologic grade (B).

Until now, classic ILC and PILC tumors were considered to be low-to-intermediate-grade and high-grade tumors, respectively. However, in the present series, 30% of classic ILC tumors are characterized as GG3 or GG equivocal, and up to 30% of PILC are characterized as GG1. Moreover, we observed 5% (9) of HER2-positive cases in the present series including seven classical ILC and 2 PILC.

In a previous restrospective analysis of 13 International Breast Cancer Study Group (IBCSG) studies, 767 ILC patients were identified and compared with 8607 IDC patients. The ILC subgroup was enriched for older patients (>50 years) and had a higher frequency of HG2 and ER-positive tumors than the IDC subgroup. In addition, IDC patients had better OS within the first 10 years after diagnosis when compared with ILC, but the opposite effect was observed with longer follow-up. Similar to the IBCSG studies, our series is mostly represented by patients older than 50, whose tumors largely classified as HG2 (72%) and ER-positive (94%). In contrast to the IBCSG series, the present study has a shorter median follow-up, and it is not clear whether GG can identify patients with a higher chance to experience a late OS event (>10 years).

Older age at diagnosis and high-ER-positivity rates have been consistently reported across different ILC series and are important factors commonly taken into consideration for adjuvant treatment decision making [20]. However, ILC tumors do not respond consistently well to chemotherapy [3], compounding the uncertainties associated with making appropriate treatment decisions for this group of patients. In our series, the benefit conferred by chemotherapy could be linked to the considerable percentage of ILC tumors

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Table 2. Multivariate Cox proportional hazard models of IDFS stratified by center

	HR	95% CI	*P*-value
Model 1: without genomic grade			
Age			
<50	1		
≥50	2.3	0.9–6.3	0.10
ILC histotype			
Classic	1		
Pleomorphic	2.1	0.8–5.3	0.12
Tumor size			
<2 cm	1		
≥2 cm	2.7	1.0–7.3	0.05
Nodal status			
Negative	1		
1–3 positive	2.0	0.8–4.9	0.12
≥4 positive	9.8	3.8–25.3	<0.0001
Histologic grade			
1	1		
2	0.6	0.3–1.3	0.18
3	0.9	0.3–3.1	0.92
HER2			
Negative	1		
Positive	2.7	1.0–7.6	0.06
Adjuvant chemotherapy			
No	1		
Yes	0.4	0.2–0.9	0.02
Model 2: with genomic grade			
Age			
<50	1		
≥50	3.2	1.1–9.2	0.03
ILC histotype			
Classic	1		
Pleomorphic	1.8	0.7–4.5	0.24
Tumor size			
<2 cm	1		
≥2 cm	3.6	1.3–10.4	0.01
Nodal status			
Negative	1		
1–3 positive	1.9	0.8–4.8	0.15
≥4 positive	10.7	4.1–28.1	<0.0001
Histologic grade			
1	1		
2	0.4	0.2–0.9	0.03
3	0.4	0.1–1.4	0.13
HER2			
Negative	1		
Positive	1.1	0.3–3.5	0.91
Adjuvant chemotherapy			
No	1		
Yes	0.4	0.2–0.9	0.02
Genomic grade			
1	1		
Equivocal	2.8	1.2–6.8	0.02
3	5.6	2.1–15.3	<0.001
		$\Delta x^2 = 12.1$	0.002

CI, confidence interval; HR, hazard ratio; ILC, invasive lobular carcinoma.
P-values correspond to Wald statistics unless specifically indicated; Δx^2 *P*-value is based on the value of adding genomic grade to the model containing all clinicopathologic variables.

Table 3. Multivariate Cox proportional hazard models of OS stratified by center

	HR	95% CI	*P*-value
Model 1: without genomic grade			
Age			
<50	1		
≥50	3.2	0.6–16.2	0.16
ILC histotype			
Classic	1		
Pleomorphic	2.3	0.5–11.5	0.30
Tumor size			
<2 cm	1		
≥2 cm	1.1	0.3–4.3	0.92
Nodal status			
Negative	1		
1–3 positive	2.7	0.5–14.3	0.23
≥4 positive	31.5	6.2–159.9	<0.0001
Histologic grade			
1	1		
2	0.6	0.2–2.4	0.49
3	0.8	0.1–5.6	0.85
HER2			
Negative	1		
Positive	0.3	0–2.8	0.30
Adjuvant chemotherapy			
No	1		
Yes	0.2	0.1–0.9	0.03
Model 2: with genomic grade			
Age			
<50	1		
≥50	4.7	0.8–27.7	0.08
ILC histotype			
Classic	1		
Pleomorphic	1.7	0.3–8.4	0.51
Tumor size			
<2 cm	1		
≥2 cm	1.4	0.3–6.4	0.68
Nodal status			
Negative	1		
1–3 positive	2.6	0.5–14.1	0.27
≥4 positive	36.9	6.5–210.3	<0.0001
Histologic grade			
1	1		
2	0.4	0.1–1.6	0.21
3	0.3	0–2.2	0.23
HER2			
Negative	1		
Positive	0.1	0–1.5	0.10
Adjuvant chemotherapy			
No	1		
Yes	0.3	0.1–0.9	0.04
Genomic grade			
1	1		
Equivocal	3.4	1.0–11.6	0.005
3	7.2	1.6–32.2	0.01
		$\Delta x^2 = 12.1$	0.002

HR, hazard ratio.
P-values correspond to Wald statistics unless specifically indicated; Δx^2 *P*-value is based on the value of adding genomic grade to the model containing all clinicopathologic variables.

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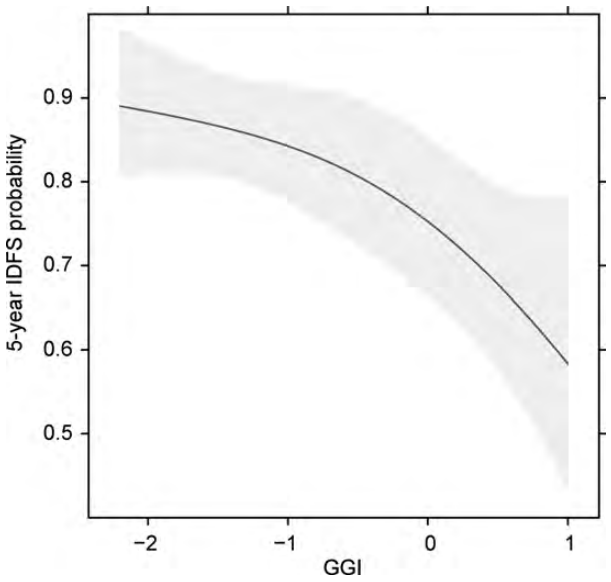

Figure 3. Predicted 5-year invasive disease-free survival as a function of continuous genomic grade index. Shaded area represents 95% CI. GGI, genomic grade index; IDFS, invasive disease-free survival.

characterized as GG equivocal and GG high, although the retrospective nature of this study limits our ability to draw definitive conclusions. In patients with IDC, the highly proliferative ER-positive disease subset determined by GG or other gene signatures represents the group most likely to benefit from adjuvant chemotherapy, while the low proliferative ER-positive group is less likely to derive such benefit [12, 21–23].

In previous studies, GG has shown its ability to categorize ER-positive IDC tumors into two subtypes with distinct clinical outcomes [24]. In a retrospective analysis including 666 tumor samples, high GGI score was significantly associated with worse survival outcome in both treated and untreated datasets [24]. A subsequent analysis of tumors from 229 patients treated with primary neoadjuvant chemotherapy demonstrated a direct association between high GGI score and better response to treatment [21].

Ki67 labeling index has also been used to stratify ER-positive breast cancer into low and highly proliferative subtypes [11, 12]. However, in our study, Ki67 evaluated either as a continuous or as a dichotomous variable failed to predict patient outcomes. Importantly, the lack of a centralized and standardized Ki67 evaluation in the present study limits definitive conclusions [25]. Moreover, the Ki67 cut point used in the present analysis was defined in a non-ILC breast cancer population, making its applicability to ILC patients uncertain.

In summary, this study represents the first attempt to evaluate the prognostic value of GG in a population of ILC, in particular with respect to HG. Similar to what we had previously determined for IDC, tumor cell proliferation evaluated through GG has important prognostic value for patients with ILC, including those with node-positive disease, adding prognostic information to classic clinicopathologic variables. The present results have shed light on the prognosis of ILC and may help guide the design of future clinical studies enrolling patients diagnosed with ILC.

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disclosures

Ipsogen SA provided financial support for the conduction of the present study but imposed no restrictions on investigators in respect to study design, data collection, data analysis, data interpretation and writing of the report. H P-S-P and AC work at Ipsogen as chief medical officer and medical affairs manager, respectively. The remaining authors have declared no conflicts of interest.

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The prognostic role of TGF-β signaling pathway in breast cancer patients

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Background: The transforming growth factor-β (TGF-β) pathway has dual effects on tumor growth. Seemingly, discordant results have been published on the relation between TGF-β signaling markers and prognosis in breast cancer. Improved prognostic information for breast cancer patients might be obtained by assessing interactions among TGF-β signaling biomarkers.

Patients and methods: The expression of nuclear Smad4, nuclear phosphorylated-Smad2 (p-Smad2), and the membranous expression of TGF-β receptors I and II (TβRI and TβRII) was determined on a tissue microarray of 574 breast carcinomas. Tumors were stratified according to the Smad4 expression in combination with p-Smad2 expression or Smad4 in combination with the expression of both TGF-β receptors.

Results: Tumors with high expression of TβRII, TβRI and TβRII, and p-Smad2 ($P = 0.018$, 0.005, and 0.022, respectively), and low expression of Smad4 ($P = 0.005$) had an unfavorable prognosis concerning progression-free survival. Low Smad4 expression combined with high p-Smad2 expression or low expression of Smad4 combined with high expression of both TGF-β receptors displayed an increased hazard ratio of 3.04 [95% confidence interval (CI) 1.390–6.658] and 2.20 (95% CI 1.464–3.307), respectively, for disease relapse.

Conclusions: Combining TGF-β biomarkers provides prognostic information for patients with stage I–III breast cancer. This can identify patients at increased risk for disease recurrence that might therefore be candidates for additional treatment.

Key words: breast cancer, prognosis, p-Smad2, smad4, TGF-β receptor I, TGF-β receptor II

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