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EndoPredict improves the prognostic classification derived from common clinical guidelines in ER-positive, HER2-negative early breast cancer

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Background: In early estrogen receptor (ER)-positive/HER2-negative breast cancer, the decision to administer chemotherapy is largely based on prognostic criteria. The combined molecular/clinical EndoPredict test (EPclin) has been validated to accurately assess prognosis in this population. In this study, the clinical relevance of EPclin in relation to well-established clinical guidelines is assessed.

Patients and methods: We assigned risk groups to 1702 ER-positive/HER2-negative postmenopausal women from two large phase III trials treated only with endocrine therapy. Prognosis was assigned according to National Comprehensive Cancer Center Network-, German S3-, St Gallen guidelines and the EPclin. Prognostic groups were compared using the Kaplan–Meier survival analysis.

Results: After 10 years, absolute risk reductions (ARR) between the high- and low-risk groups ranged from 6.9% to 11.2% if assigned according to guidelines. It was at 18.7% for EPclin. EPclin reassigned 58%–61% of women classified as high-/intermediate-risk (according to clinical guidelines) to low risk. Women reclassified to low risk showed a 5% rate of distant metastasis at 10 years.

Conclusion: The EPclin score is able to predict favorable prognosis in a majority of patients that clinical guidelines would assign to intermediate or high risk. EPclin may reduce the indications for chemotherapy in ER-positive postmenopausal women with a limited number of clinical risk factors.

Key words: adjuvant treatment, breast cancer, endocrine therapy, EndoPredict gene, expression

introduction

Breast cancer patients with estrogen receptor (ER)-positive, HER2-negative disease have a significant clinical benefit from adjuvant endocrine therapy [1, 2]. The benefit of systemic chemotherapy for the individual woman remains uncertain due to the absence of validated predictive markers concerning cytotoxic treatment in this largest subset of early breast cancer patients [3].

The current goal in daily clinical decision-making is to accurately define individual prognosis. If it is possible to assign a risk of breast cancer recurrence under adjuvant endocrine therapy, which is very low or favorable in relation to the competing health risks, then the additional value of chemotherapy will have to be considered cautiously with the patient. Current clinical guidelines incorporate clinical and pathological factors such as tumor size, grading, and nodal status for the decision whether to combine chemotherapy and endocrine treatment. However, today's guidelines identify only a small subset of patients, with such a low risk to justify treatment with endocrine therapy alone [4, 5]. It has been proposed that there may be an overtreatment of adjuvant breast cancer patients using clinicopathological parameters for risk stratification [6].

Since 2009, the St Gallen International breast cancer panel has recognized both the robustness of validated gene

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expression tests and their ability to add prognostic information to clinicopathological factors [7]. In 2011, the panel expanded on this first step and recommended a classification reflecting intrinsic properties of the tumor in order to improve clinical risk stratification and allow for more informed decisions on a tailored treatment strategy [5].

In ER-positive and HER-2 negative tumors, stratification in Luminal A or Luminal B has been recommended in order to better clarify the indication for chemo-endocrine therapy [5]. This stratification however is dependent on a reliable assessment of grading and/or Ki67 measured immunohistochemically. Unfortunately, both variables suffer from considerable interobserver variability [8]. As a consequence, validated multigene tests have gained clinical importance since they may add robustness and better reflect the intrinsic biology and therefore prognosis of breast cancer [5, 7].

Several multigene algorithms have been developed to estimate the individual risk of recurrence [9–12]. Recently, the EndoPredict has been introduced as a novel multigene test for predicting the likelihood of distant recurrence in patients with ER-positive, HER2-negative breast cancer treated with adjuvant endocrine therapy only [13]. The EP is based on the quantification of mRNA levels of eight genes of interest in formalin-fixed, paraffin-embedded tissue sections by quantitative RT-PCR (qRT-PCR). The combination of the EP with the two clinical risk factors nodal status and tumor size resulted in the EPclin. It could identify subgroups showing remarkable differences in 10-year distant recurrence rates in two large randomized phase III trials [Austrian Breast and Colorectal Cancer Study Group (ABCSG)-6 and ABCSG-8]. Additionally, the EP/EPclin classifier is the first test that substantially adds prognostic information to all common clinicopathological parameters, including Ki67 staining [13]. Thus, EPclin is able to delineate a subgroup of postmenopausal women with an extremely low risk of distant recurrence irrespective of, e. g. grading or proliferative index. Recently, it has been shown that the EndoPredict test can be successfully implemented in molecular pathological routine laboratories and is feasible for reliable decentralized assessment of gene expression in luminal breast cancer [14].

The purpose of this study was to evaluate the practical improvements a clinical use of the EPclin may confer to risk classification of breast cancer patients after considering common clinicopathological guidelines. Three common guidelines or recommendations—American National Comprehensive Cancer Center Network (NCCN) 2007, German S3 2008 and St Gallen 2011—were selected and the concordance and performance were retrospectively analyzed compared with the EPclin. A total of 1702 ER-positive, HER2-negative postmenopausal breast cancer patients were selected from two large randomized phase III trials ABCSG-6 and ABCSG -8. To assess the clinical relevance of the EPclin test, we explored whether the EPclin could be used to stratify patients more accurately than the common clinical guidelines.

patients and methods

patients, samples, statistics

Patients included in this study participated in the ABCSG-6 (tamoxifen-only arm) or ABCSG-8 trial [15–17]. They received either tamoxifen for 5 years or tamoxifen for 2 years followed by anastrozole for 3 years (patients characteristics—supplementary Table S1 and supplemental methods, available at *Annals of Oncology* online). All 1702 ER-positive, HER2-negative breast cancer patients were retrospectively assigned to risk categories based on the EPclin and on the German S3, St Gallen and American NCCN guidelines. The median follow-up time was 63 months for the combined cohort.

The details about the statistical analysis are described in supplementary methods, available at *Annals of Oncology* online.

EPclin

Combining EP (detailed description in supplementary methods, available at *Annals of Oncology* online) with the two clinical risk factors nodal status and tumor size results in the EPclin. EPclin low-risk and high-risk categories were prespecified before the validation in the ABSCG-6 and ABSCG-8 studies, as recently described [13]. Patients with an EPclin score <3.3 were classified as low risk for distance recurrence, whereas patients with an EPclin score ≥3.3 were stratified as high risk. Thus, the cohort analyzed in this article represents the validation but not the training set for EPclin.

German S3 guidelines 2008

In accordance with the St Gallen Recommendations 2005/2007, the German S3 guidelines classify patients with a tumor <2 cm with a well-differentiated phenotype (grade 1) and no positive lymph nodes as low risk. Patients were classified as intermediate-/high-risk in case of a tumor size >2 cm and/or grade ≥2 and/or lymph node involvement.

NCCN guidelines

NCCN 2007 guidelines were recently used to compare the performance of a multigene test with clinical risk stratification [18]. We used the NCCN risk categorization from 2007, because the current guidelines are not solely based on clinicopathological factors that are commonly available.

Patients were divided into two risk groups based on the NCCN 2007 guidelines: tumors ≤0.5 cm with no involved lymph nodes were classified as low risk. Additionally, tumors with grade 1 and a tumor size ≤1.0 cm and no involved lymph nodes were also classified as low risk. Patients with a tumor of grade 2/3 and a tumor size between 0.6 and 1.0 cm were classified as high risk. Additionally, tumors with a tumor size >1.0 cm (independent of grading) were stratified as high risk.

St Gallen consensus recommendations 2011

Patients were stratified according to the recent St Gallen consensus recommendations [5]. Briefly, immunohistochemically determined Ki67 staining was used to distinguish between the biological breast cancer subtypes 'Luminal A' and 'Luminal B'. Tumors with a Ki67 staining <14% ('Luminal A') [19] and a well-differentiated phenotype (grade 1) and less than four involved lymph nodes were classified as low risk. 'Luminal B' tumors and 'Luminal A' tumors with a grade 2/3 and/or more than three involved lymph nodes were classified as intermediate/high risk.

results

risk stratification according to clinical guidelines and the EPclin in 1702 ER-positive, HER2-negative postmenopausal breast cancer patients

To assess the impact of the EPclin on risk stratification, we retrospectively analyzed 1702 ER-positive, HER2-negative postmenopausal breast cancer patients from the ABCSG-6 and -8 trials (patient characteristics—supplementary Table S1, available at *Annals of Oncology* online). All patients were assigned to risk categories according to the prespecified EPclin cut-off value and the German S3, NCCN and St Gallen treatment recommendations.

Fifteen percent of all patients were classified as low risk according the German S3 guidelines. The NCCN guidelines classified only 6% of all patients as low risk and St Gallen criteria assigned 19% of women to a low-risk group. (supplementary Table S2, available at *Annals of Oncology* online), EPclin attributed low risk to 63% of all women analyzed.

In the Kaplan–Meier analysis, all stratifiers were able to delineate a group of patients with extremely low risk: S3 low risk showed an absolute freedom of distance recurrence of 94.7% (90.5%–98.9%) after 10 years of follow-up. NCCN, St Gallen and EPclin showed a very similar result with 94.5% (88.9%–100%), 96.9% (94.9%–98.9%) and 95.3% (93.4%–97.3%), respectively.

In order to assess the difference in distant recurrence-free survival of low-risk groups versus intermediate-/high-risk patients, log-rank tests, hazard ratios and absolute risk reductions (ARR) at 10 years of follow-up were calculated. The German S3 low-risk group showed significantly better metastasis-free survival (MFS) than the intermediate-/high-risk group [$P = 0.014$, HR = 2.20 (1.16–4.19), ARR = 7.9% (3.0%–12.9%), Figure 1A], whereas there was no significant difference for the NCCN risk categorization [$P = 0.12$, HR = 2.16 (0.80–5.85), ARR = 6.9% (0.9%–13%), Figure 1B). According to the recent St Gallen recommendations, the low-risk classification was significantly associated with increased 10-year distant MFS [$P < 0.001$, HR = 2.78 (1.50–5.14), ARR = 11.2% (7.7%–14.7%),

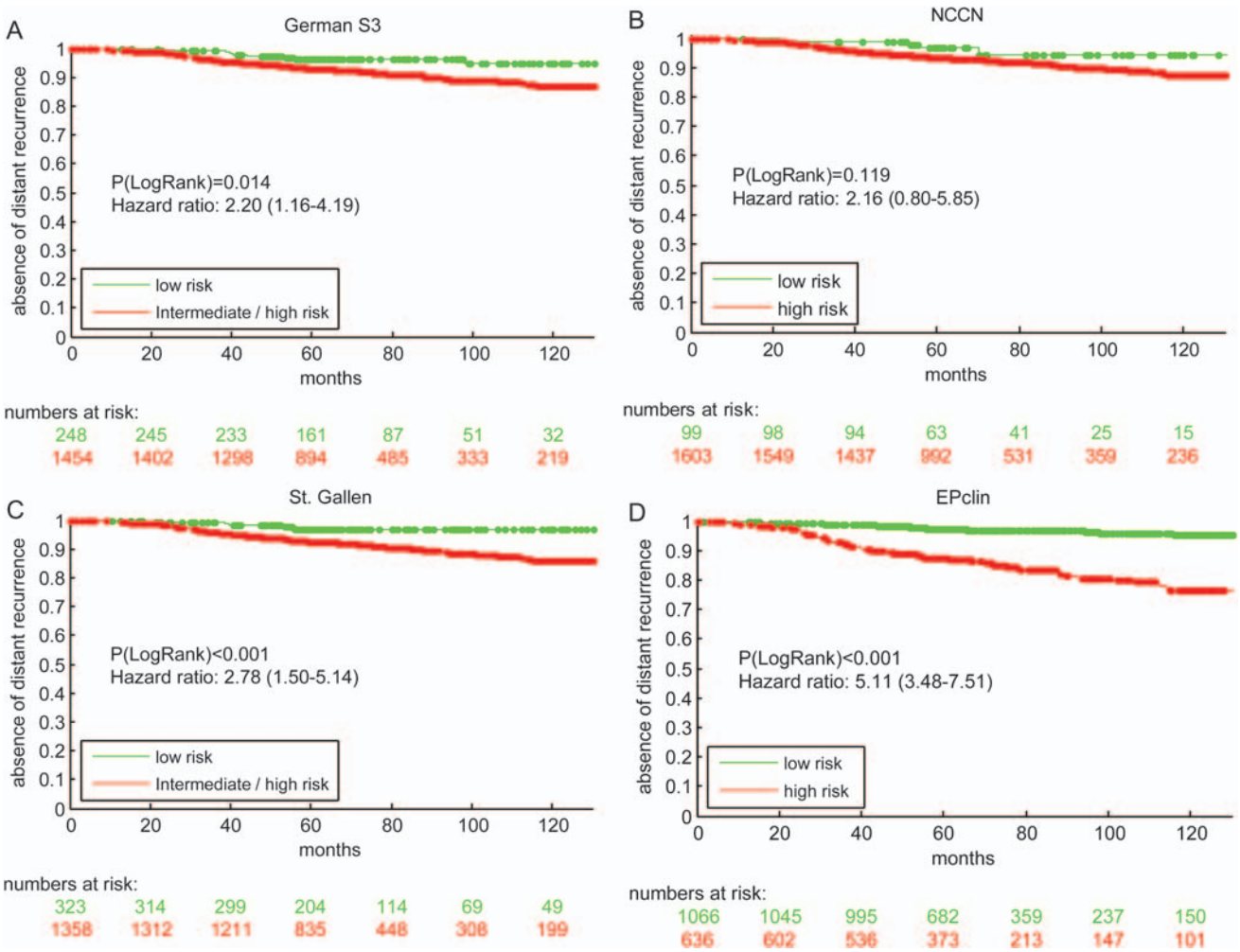

Figure 1. Kaplan–Meier plot of distant metastasis-free survival (MFS) by (A) German S3, (B) National Comprehensive Cancer Center Network (NCCN), (C) St Gallen guidelines and (D) EPclin risk groups. 95% confidence intervals (CI) of hazard ratios (HR) are indicated.

Figure 1C]. The combined molecular/clinicopathological EPclin resulted in the best separation between the low- and high-risk groups [$P < 0.001$, 5.11 (3.48–7.51), ARR = 18.7% (13.5%–23.9%), Figure 1d]. In summary, prognostic categorizations using clinical guidelines identify a subgroup with a remarkably low rate of distant metastasis. However, the molecular test showed greater classification accuracy and accordingly delivered a greater absolute difference in distant recurrence between the risk groups: The ARR was 18.7% for the EPclin, resulting in an improved absolute difference in distant recurrence between the risk groups of 7.5%–11.8% in comparison to the clinical guidelines

analysis of concordance and discordance between molecular and clinical risk stratification

To analyze the impact of the EPclin-based risk stratification in detail, we compared its performance with the three selected clinical guidelines. First, we examined the concordance of the respective risk categorization between the EPclin and the German S3, NCCN and St Gallen guidelines (Table 1). The vast majority (82%–94%) of all clinically assigned low-risk patients were also classified as low risk by the EPclin. Given the large overlap and the low number of metastatic events in the low-risk group, the EPclin was not significantly associated with better MFS for the low-risk groups after considering the German S3, NCCN and St Gallen guidelines (data not shown).

In contrast, the majority of patients deemed intermediate/high risk by the German S3 guidelines were reclassified as low risk by the EPclin (841 of 1454, 58%, Table 1; supplementary Table S3, available at *Annals of Oncology* online). A comparable reclassification was also observed for the NCCN-based high-risk group (973 of 1603, 61%, Table 1) and the St Gallen intermediate-/high-risk group (782 of 1358, 58%, Table 1).

All patients stratified as intermediate or high risk by clinical guidelines were stratified by EPclin in the Kaplan-Meier analysis (Figure 2). This was to test if the reclassification by EPClin was corresponding to actual distant recurrence-free survival of the patients. At 10 years, the distant recurrence rates for patients with EPclin-low risk and EPclin-high risk were 5% (2%–7%) and 24% (19%–29%) in the German S3 intermediate-/high-risk group (Figure 2a). Comparable results were observed, when S3-high-risk patients ($n = 83$) were omitted from the analysis: The distant recurrence rates for patients with EPclin-low risk and EPclin-high risk were 5% (2%–7%) and 20% (15%–25%) in the German S3 intermediate-risk group (data not shown).

Additionally, the EPclin-based classification resulted in distant recurrence rates for patient with EPclin-low risk and EPclin-high risk of 5% (3%–7%) and 23% (18%–28%) in the NCCN high-risk group (Figure 2b), and 5% (3%–8%) and 25% (20%–30%) in the St Gallen intermediate-/high-risk group (Figure 2c), respectively.

St Gallen 2011 recommendations—performance of the EPclin in Luminal A and Luminal B subtypes

The St Gallen 2011 panel recommended an immunohistochemically based classification as a 'shorthand' for the molecular Luminal A and Luminal B intrinsic breast cancer subtypes. In this surrogate system, the 'Luminal B' subtype is identified by a high Ki67 staining index (Ki67 >14%); in the absence of Ki67 labeling index, grading has been recommended.

We analyzed the performance of the EPclin in Ki67 high (>14%), low and G1, 2, 3 tumors of our cohort. According to the EPclin categorization, 34% of all 'Luminal B' tumors were classified as low risk. Interestingly, 29% of all 'Luminal A' tumors were reclassified as high risk according to EPclin. The EPclin-based risk stratification was significantly associated with improved MFS in both biological subtypes (Figure 3a and b, supplementary Table S4, available at *Annals of Oncology* online). Additionally, we analyzed the EPclin-based risk stratification after considering the grading status. The EPclin classified 78% of all grade 1 tumors, 60% of all grade 2 tumors and 19% of all grade 3 tumors as low risk. All low-risk groups had an excellent MFS (Figure 4a–c, supplementary Table S5, available at *Annals of Oncology* online).

discussion

In our study, we show that three widely regarded guidelines and the combined molecular and clinical predictor EPclin are able to identify a subset of ER-positive, HER-2 negative, postmenopausal breast cancer patients with excellent prognosis when treated with endocrine therapy in the absence of

Table 1. Comparison between EPclin and German S3, National Comprehensive Cancer Center Network (NCCN) 2007 and St Gallen 2011 based risk stratification

German S3	German S3 low ($n = 248$, 14.6%)	German S3 intermediate/high ($n = 1454$, 85.4%)
EPclin low ($n = 1066$, 62.6%)	225 (13.2%)	841 (49.4%)
EPclin high ($n = 636$, 37.4%)	23 (1.4%)	613 (36.0%)
National Comprehensive Cancer Center Network (NCCN) 2007	NCCN low ($n = 99$, 5.8%)	NCCN high ($n = 1603$, 94.2%)
EPclin low ($n = 1066$, 62.6%)	93 (5.5%)	973 (57.2%)
EPclin high ($n = 636$, 37.4%)	6 (0.4%)	630 (37.0%)
St Gallen 2011	St Gallen low ($n = 323$, 19.2%)	St Gallen intermediate/high ($n = 1358$, 80.8%)
EPclin low ($n = 1048$, 62.3%)	266 (15.8%)	782 (46.5%)
EPclin high ($n = 633$, 37.7%)	57 (3.4%)	576 (34.3%)

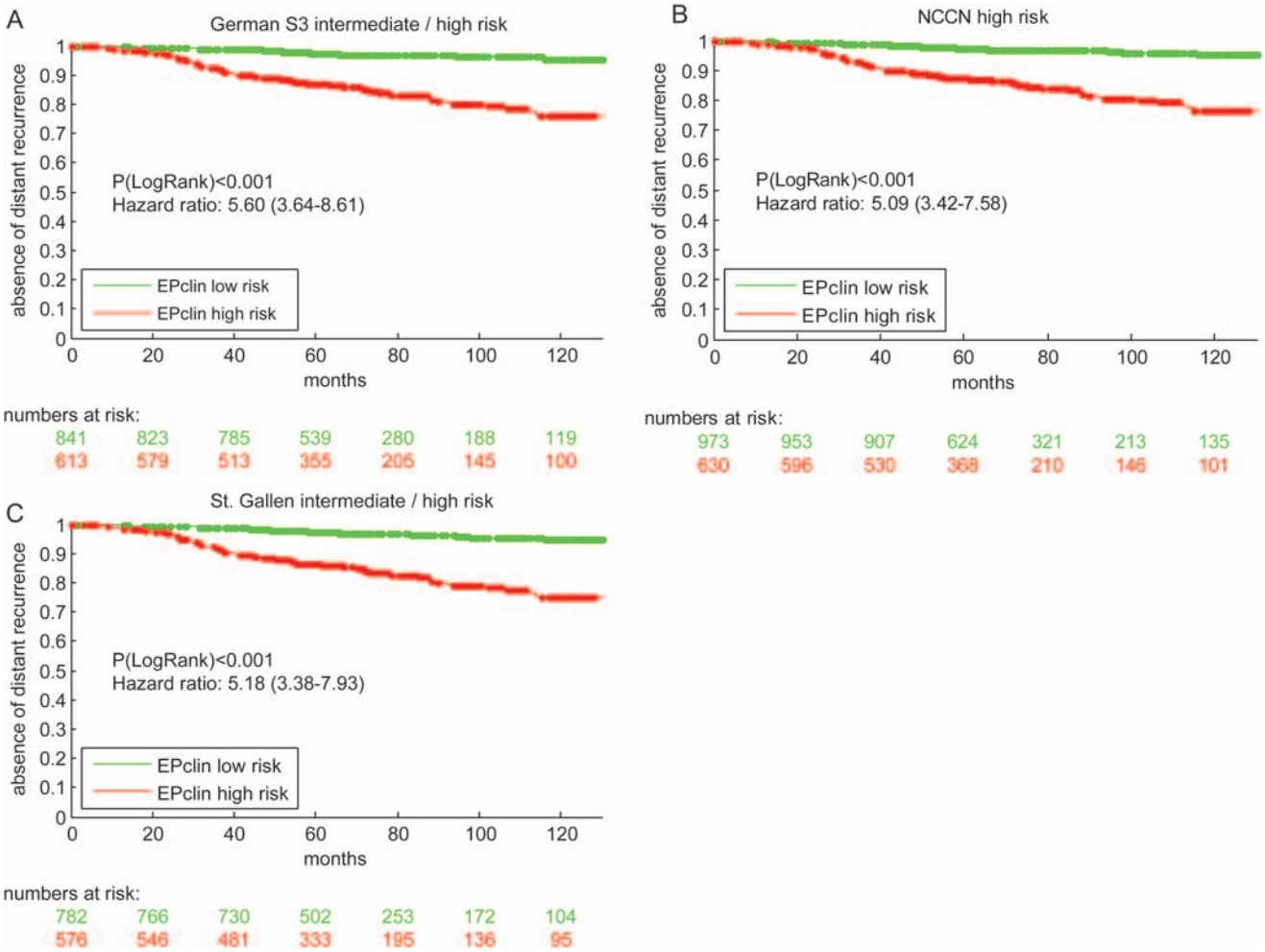

Figure 2. Kaplan–Meier plot of distant metastasis-free survival (MFS) by EPclin risk groups after considering the (A) German S3, (B) National Comprehensive Cancer Center Network (NCCN) and (C) St Gallen guidelines.

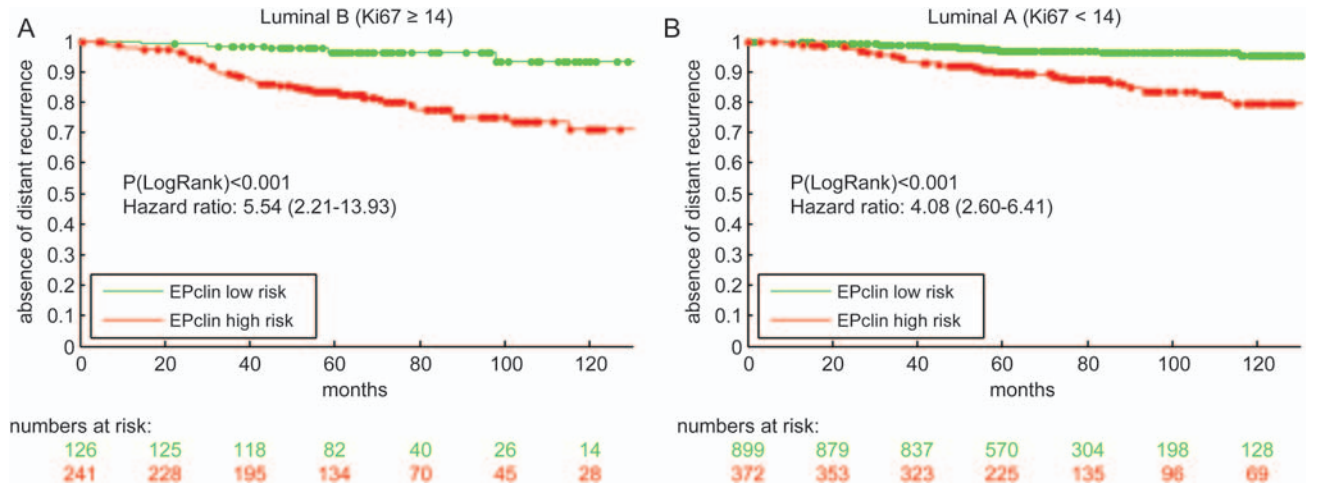

Figure 3. Kaplan–Meier plot of distant metastasis-free survival (MFS) by EPclin risk groups after considering the biological subtypes (A) 'Luminal B' and (B) 'Luminal A'.

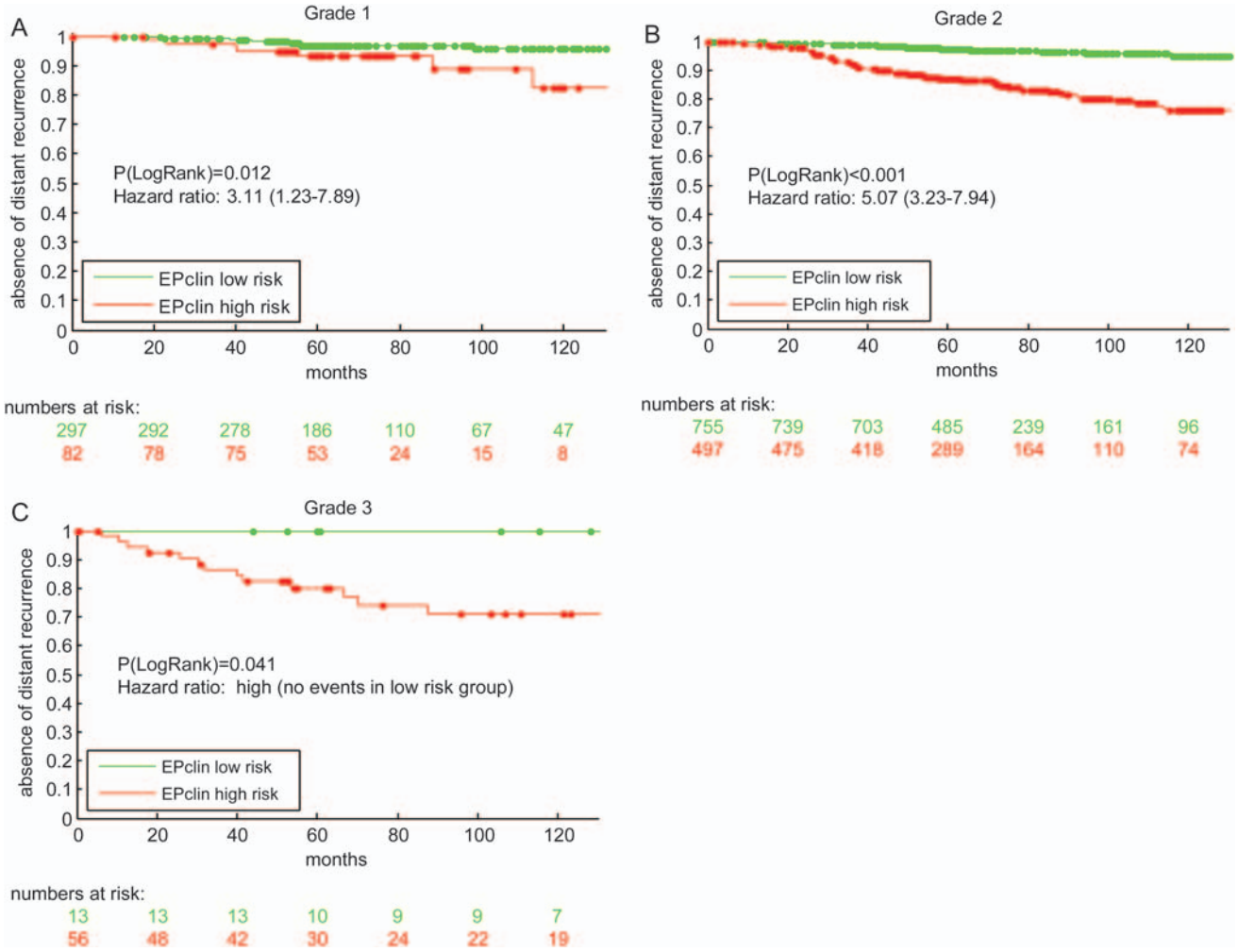

Figure 4. Kaplan–Meier plot of distant metastasis-free survival (MFS) by EPclin risk groups in (A) grade 1 tumors, (B) grade 2 tumors and (C) grade 3 tumors.

chemotherapy. These patients have a rate of distant recurrence of ~5% after 10 years of follow-up. We further show that EPclin is able to assign this type of survival to almost two-third of this breast cancer cohort, whereas stratification according to guidelines will assign low risk only to a minority of patients. This increase in classification accuracy is mostly due to a reclassification of clinically intermediate to high-risk patients to EPclin-low risk. Furthermore, we establish that the surrogate Luminal A cohort (defined according to the St Gallen 2011 recommendations) includes close to a third of women with elevated risk.

We retrospectively analyzed a combined cohort from the ABCSG-6 and ABCSG-8 phase III trials encompassing 1702 ER-positive, HER2-negative tumors treated with endocrine therapy (TAM for 5 years, or 2 years of TAM followed by 3 years of ANA) only. It is important to point out that clinicians selected these patients for randomization in two trials only employing endocrine treatment in the absence of chemotherapy. Both the full cohorts and the biomarker cohorts thus display a limited amount of clinical risk factors such as high grading, positive nodal status or larger tumors and this limits the statistical power of the study in these subsets. Thus, the strength of this study is to assign 'molecular' risk in clinically intermediate to low-risk patients and show association with actual outcome. Due to the low number of clinically high-risk patients, especially patients with G3 tumors, the analyses in these subgroups should be regarded as exploratory.

Ki67 immunohistochemical staining has been a matter of discussion because different cut-off values have been used to identify the 'Luminal B' subtype characterized by a high proliferation [20, 21]. Cheang et al. recently compared the molecular subtyping by PAM50 [22] with Ki67 staining and identified an optimal threshold for immunohistochemistry of 13.25% [19]. Nevertheless, the false-positive and false-negative rates for the detection of the intrinsic molecular subtypes were high suggesting that gene expression profiling is more reliable than a single immunohistochemical marker [23]. In our study, we used the Ki67 cut-off level that was recommended by the St Gallen panel [5]. Tumors with intermediate or high grading were classified in the intermediate-/high-risk group despite low Ki67. We have chosen this interpretation of the St Gallen

guidelines to reflect the uncertain quality of the biomarker Ki67 to distinguish between Luminal A and Luminal B tumors. To further analyze the effect of the EPclin classification in the context of Ki67/grading classification in detail, we applied EPclin-based risk categorization in Ki67 and grade subclasses.

The results show that a molecular test in combination with defined clinical factors may encompass more of the tumors' (and possibly the hosts') intrinsic biology than the classic assumption based on clinicopathological factors is able to cover.

ER-positive, HER2-negative postmenopausal patients constitute the largest subset of breast cancer patients. The Luminal A and B terminology refers to proliferative and prognostic phenotypes. The driving mutational patterns and pathway alterations, however, are likely to be very diverse [24]. The quest to establish validated predictive factors for cytotoxic therapy (and its failure) has demonstrated many levels of complexity involved; factors including not only tumors but also pharmacodynamics of treatment, environment and most of all tumor–host treatment interactions. Thus, at the end of the day, clinical routine is limited to making the best possible estimation of prognosis and predict a possible benefit of cytotoxic treatment on that basis.

What are the clinical implications of these results? A large group of patients will have concordant results: both clinical factors and the EPclin will point to either high or low risk, making treatment decisions fairly clear. A very rare clinical scenario with discordant results would suggest favorable prognosis in view of clinical factors and an EPclin score indicating high risk. The addition of, e.g. cytotoxic therapy to these cases (or possibly molecular therapies in the future) currently lacks prospective evidence. Furthermore, clinical scenarios may arise where a clear-cut indication for chemotherapy (e.g. more than three involved lymph nodes, ER poor and G3) may be associated with a low EPclin score. In these cases, we are currently reluctant to follow the molecular advice. The validation cohort (patients randomly assigned to endocrine therapy in the absence of adjuvant chemotherapy; ABCSG 6 and 8) only showed a small number of patients with such clinical features. Indeed, the indication to perform a molecular test in such a patient is questionable.

The most frequent clinical scenario will be patients with one or two factors that may suggest higher risk: e.g. G2 or lymphovascular invasion, or a single-positive lymph node and Ki67>14%. In these scenarios where a single to several relative indications for chemotherapy may be present, the validation of the EPclin in the described cohort is well suited to add prognostic information. This study demonstrates that 47%–57% of all women assigned to intermediate/high risk by common clinical guidelines could be spared chemotherapy (Figure 5). This assessment adds to the evidence that the combined molecular and clinical test is more sensitive in describing a good prognosis group of women.

In summary, this study indicates that almost two-thirds of ER-positive postmenopausal women with a limited number of risk factors can be assigned an excellent prognosis using the combined molecular and clinical test EPclin. In comparison to widely used treatment recommendations, this represents a more exact estimation of prognosis and will improve the choices in administering tailored adjuvant treatment in ER-positive breast cancer.

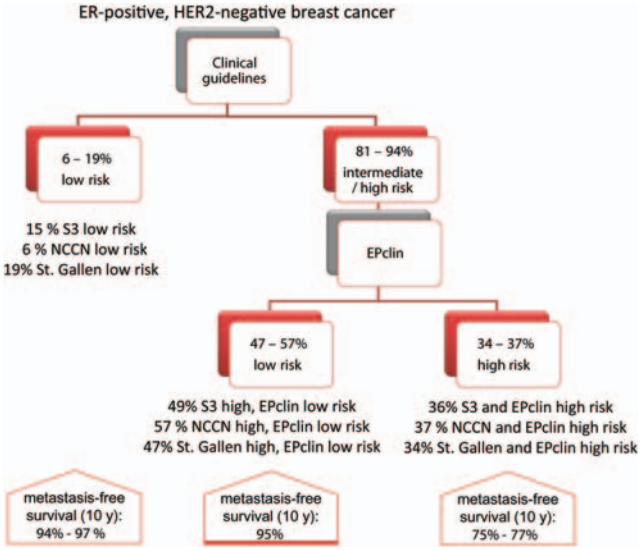

Figure 5. Putative risk classification scheme for estrogen receptor (ER)-positive, HER2-negative breast cancer patients by combining clinical guidelines and the EPclin test. Metastasis-free survival (MFS) rates are based on the 1702 ER-positive, HER2-negative breast cancer samples.

disclosures

PD and MF have received honoraria from Sividon Diagnostics GmbH for lectures concerning EP. CP, KEW, RK and JCB are employees of Sividon Diagnostics GmbH. CP, KEW and RK are shareholders of Sividon Diagnostics GmbH. The other authors have declared no conflicts of interest.

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