CLINICAL TRIAL

Prognostic effect of Ki-67 in common clinical subgroups of patients with HER2-negative, hormone receptor-positive early breast cancer

Peter A. Fasching[1,2] · Paul Gass[1] · Lothar Häberle[1,3] · Bernhard Volz[1] · Alexander Hein[1] · Carolin C. Hack[1] · Michael P. Lux[1] · Sebastian M. Jud[1] · Arndt Hartmann[4] · Matthias W. Beckmann[1] · Dennis J. Slamon[2] · Ramona Erber[4]

Abstract

Purpose Several clinical trials have investigated the prognostic and predictive usefulness of molecular markers. With limited predictive value, molecular markers have mainly been used to identify prognostic subgroups in which the indication for chemotherapy is doubtful and the prognosis is favorable enough for chemotherapy to be avoided. However, limited information is available about which groups of patients may benefit from additional therapy. This study aimed to describe the prognostic effects of Ki-67 in several common subgroups of patients with early breast cancer.

Methods This retrospective study analyzed a single-center cohort of 3140 patients with HER2−, hormone receptor-positive breast cancer. Five-year disease-free survival (DFS) rates were calculated for low ($< 10\%$), intermediate (10–19%), and high ($\geq 20\%$) Ki-67 expression levels, as assessed by immunohistochemistry, and for subgroups relative to age, body mass index, disease stage, tumor grade, and (neo-)adjuvant chemotherapy. It was also investigated whether Ki-67 had different effects on DFS in these subgroups.

Results The 5-year DFS rates for patients with low, intermediate, and high levels of Ki-67 expression were 0.90, 0.89, and 0.77, respectively. Ki-67 was able to further differentiate patients with an intermediate prognosis into different prognostic groups relative to common clinical parameters. Patients with stage II breast cancer had 5-year DFS rates of 0.84, 0.88, and 0.79 for low, intermediate, and high levels of Ki-67 expression. Ki-67 had different prognostic effects in subgroups defined by age and tumor grade.

Conclusions Ki-67 may help identify patients in intermediate prognostic groups with an unfavorable prognosis who may benefit from further therapy.

Keywords Breast cancer · Ki-67 · Proliferation · Molecular marker · Prognosis

Introduction

Cell proliferation is a central process that is connected to pathogenesis and progression across cancer histologies [1, 12, 28, 30, 45]. Particularly in patients with HER2− and hormone receptor-positive breast cancer, the proliferation index

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Peter A. Fasching and Paul Gass contributed equally to this study.

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✉ Peter A. Fasching
peter.fasching@uk-erlangen.de

1 Department of Gynecology and Obstetrics, Comprehensive Cancer Center Erlangen-EMN, Erlangen University Hospital, Friedrich Alexander University of Erlangen-Nuremberg, Nuremberg, Germany

2 Division of Hematology/Oncology, Department of Medicine, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, USA

3 Biostatistics Unit, Department of Gynecology and Obstetrics, Comprehensive Cancer Center Erlangen-EMN, Erlangen University Hospital, Friedrich Alexander University of Erlangen-Nuremberg, Nuremberg, Germany

4 Comprehensive Cancer Center Erlangen-EMN, Institute of Pathology, Erlangen University Hospital, Friedrich Alexander University of Erlangen-Nuremberg, Nuremberg, Germany

is able to identify women with early and advanced stages of the disease who have an unfavorable prognosis [7, 12, 17, 29, 35, 36, 46]. Proliferation has also been included in most multigene assays for patients with early breast cancer, as one of the major driving factors that determine the prognostic value of these tests.

Patients with breast cancer who have a high proliferation index and an unfavorable prognosis usually receive chemotherapy. However, especially in the neoadjuvant setting, it has been found that the prognosis-improving effect of a pathological complete response (pCR) after chemotherapy is stronger in some subgroups than in others [11, 17, 18, 43]. pCR is an indicator of a very good prognosis both in patients with HER2+ breast cancer and in those with triple-negative breast cancer, whereas in patients with HER2−, hormone receptor-positive cancer, the effect is much smaller. However, it has been shown that Ki-67 is a very good predictor of the prognosis in the latter subgroup [17]. It therefore appears to be a promising marker to use in decision-making for or against chemotherapy in this group of patients [23], and has been included in national and international guidelines and current therapeutic studies [40, 47, 48].

CDK4/6 inhibitors (CDK4/6is) in combination with antiendocrine treatments have recently been introduced for the treatment of advanced breast cancer [19–21, 24, 33, 34, 41]. They interfere with the cell cycle and have a direct effect on proliferation. It has been shown that they lead to consistent improvement in prolonging the progression-free survival (PFS), with hazard ratios (HRs) of around 0.5 to 0.6 [19, 21, 24, 33, 34, 41]. CDK4/6is may possibly even lead to an overall survival benefit [42]. The safety profile also allows for long treatment periods [14]. The treatment might, therefore, be suitable for improving the prognosis in the adjuvant setting as well, and studies in this therapy setting are underway [8–10]. Some of these trials use Ki-67 as an inclusion criterion, and it would, therefore, be beneficial to understand the prognostic effects of Ki-67 in the various commonly used subgroups of patients with early breast cancer.

Patients and methods

Patient selection

The patients included in this retrospective study were selected from all patients with invasive breast cancer treated at the University Breast Center for Franconia, Germany, since 2001. For inclusion, the patients had to have a HER2− and hormone receptor-positive tumor, along with information available regarding staging and other covariates. Figure 1 shows a flow diagram for patient selection. Approval for carrying out the planned analysis was obtained

from the Ethics Committee of the Medical Faculty of Friedrich Alexander University, Erlangen.

Data collection

As part of a continuous quality-assurance process, each certified breast cancer center in Germany is required to document all primary diagnoses, patient and tumor characteristics, tumor board decisions, and treatment characteristics, as well as follow-up data for disease-free survival (DFS) and overall survival [3, 47]. These data have to be collected prospectively and are audited on an annual basis by the German Cancer Society (*Deutsche Krebsgesellschaft*) and German Society for Breast Diseases (*Deutsche Gesellschaft für Senologie*). As part of this process, all histopathological data have to be documented, including tumor size; axillary lymph-node status; tumor grade; and estrogen receptor, progesterone receptor, and HER2 status, among other items. The data have to be documented from the original pathology reports. All of the data used in this retrospective analysis were obtained from this database.

Histopathological data

All histopathological information used in this analysis was directly documented from the original pathological reports, which were reviewed by two investigators. Grading, tumor type, estrogen receptor status, progesterone receptor status, HER2 status, and proliferation status as assessed by Ki-67 staining have been routinely recorded at the Breast Center since 1995. The pretreatment core biopsies were stained in clinical routine as follows: estrogen receptor-alpha (clone 1D5, 1:200 dilution or clone EP1, dilution 1:40; DAKO, Denmark); progesterone receptor (clone pgR636, 1:200 dilution; DAKO, Denmark), Ki-67 (clone MIB-1, 1:200 or 1:100 dilution; DAKO, Denmark). The percentage of positively stained cells was stated in the pathological reports.

The tumors were considered to be positive for the estrogen and progesterone receptors if a percentage of at least 10% (up to 2009) or at least 1% (since 2010) more cells stained positive.

To assess HER2 status, a polyclonal antibody against HER2 (1:200 or a 1:1000 dilution, DAKO, Denmark) was used and HER2 status was stated as negative, 0, 1+, 2+, or 3+ in accordance with Sauter et al. [32]. Tumors with a score of 0 or 1+ were considered HER2−, and 3+ tumors were considered positive. Tumors with 2+ staining were tested for gene copy numbers using chromogene in situ hybridization. Using a kit with two probes of different colors (ZytoDot, 2C SPEC HER2(ERBB2)/CEN17; Zyto Vision Ltd., Bremerhaven, Germany), the gene copy numbers of HER2 and centromeres of the corresponding chromosome 17 were retrieved. A HER2/CEN17 ratio of ≥ 2.2 up to 2013
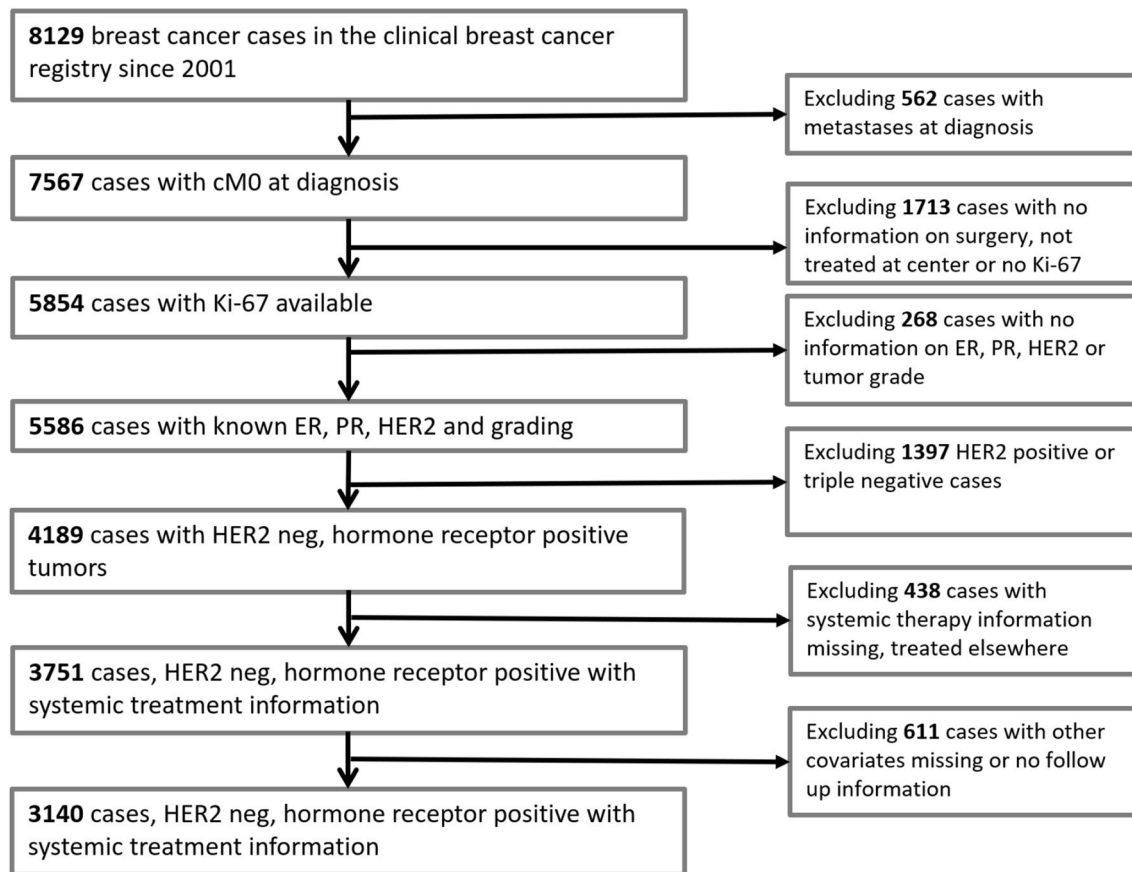
Fig. 1 Patient selection

and ≥ 2 thereafter was considered to represent amplification of HER2.

With regard to Ki-67 assessment, the overall tumor area was reviewed including the invasion front. In case there were regions with the higher percentage of Ki-67+ nuclei ("hot spot") this area determined the Ki-67 assessment.

Statistical analysis

The primary objective of this study was to analyze the effect of Ki-67 levels on DFS in commonly analyzed subgroups of patients. DFS was defined as the time from diagnosis to the earliest date of disease progression (distant metastasis, local recurrence, death from any cause) or the date of censoring, after 10 years at the latest. Survival rates, and specifically 5-year survival rates with 95% confidence intervals (CIs), were estimated using the Kaplan–Meier product limit method.

P values for interactions were calculated to assess whether the effect of Ki-67 on DFS varied between patient subgroups, using Cox regression models (one for each patient or tumor characteristic), with patient/tumor characteristic, Ki-67, and the interaction between the patient/tumor characteristic and Ki-67 as predictors. All patient and tumor characteristics were used as categorical or ordinal variables: age at diagnosis (<45 vs. 45–54 vs. 55–64 ≥ 65 years), body mass index (BMI; <25 vs. 25–25.99 vs. ≥ 30 kg/m^2), pT (1 vs. 2 vs. 3 vs. 4), pN (negative vs. positive), American Joint Committee on Cancer, AJCC stage (I vs. II vs. III), tumor grade (G1 vs. G2 vs. G3), adjuvant or neoadjuvant chemotherapy administered (no vs. yes). Ki-67 was categorized into three groups, $<10\%$, 10–19%, and $\geq 20\%$, to obtain information about subgroups similar to those used in other reports [49].

In addition, the prognostic effect of all predictors together was described using a multivariate Cox model with all patient and tumor characteristics of interest as predictors. Information about tumor size and nodal status was used, such as the AJCC stage.

All of the tests were two-sided, and a P value of <0.05 was regarded as statistically significant. Calculations were carried out using the R system for statistical computing (version 3.0.1; R Development Core Team, Vienna, Austria, 2013).

Results

A total of 3140 patients with HER2−, hormone receptor-positive breast cancer were available for the analysis. Of these, 1095 had Ki-67 levels < 10% (low), 1117 were assessed as having a Ki-67 level of 10–19% (intermediate), and 1195 cases had a Ki-67 level ≥ 20% (high).

Associations between these groups of Ki-67 and patient and tumor characteristics are shown in Table 1. Most of the tumors with Ki-67 < 10% were in stage I (n = 693, 63.3%). Tumors with Ki-67 levels of 10–19% and Ki-67 levels ≥ 20% represented stage I disease in 51.5% and 44.8% of the patients, respectively. Higher proliferation rates as assessed by Ki-67 were strongly correlated with larger tumors with a higher grade. In addition, patients

Table 1 Patient and tumor characteristics relative to Ki-67 categories, showing frequencies and percentages

| Characteristics	Ki-67 < 10% (n = 1095)	Ki-67 ≥ 10% and < 20% (n = 1117)	Ki-67 ≥ 20% (n = 1195)
Age at diagnosis (years)			
< 45	98 (8.9)	101 (9.0)	186 (15.6)
45–54	240 (21.9)	274 (24.5)	290 (24.3)
55–64	348 (31.8)	288 (25.8)	305 (25.5)
≥ 65	409 (37.4)	454 (40.6)	414 (34.6)
BMI (kg/m^2)			
< 25	486 (44.4)	476 (42.6)	519 (43.4)
25–30	397 (36.3)	404 (36.2)	382 (32.0)
≥ 30	212 (19.4)	237 (21.2)	294 (24.6)
T			
1	751 (68.6)	661 (59.2)	581 (48.6)
2	286 (26.1)	357 (32.0)	495 (41.4)
3	38 (3.5)	66 (5.9)	65 (5.4)
4	20 (1.8)	33 (3.0)	54 (4.5)
N			
pN0	859 (78.4)	773 (69.2)	721 (60.3)
pN+	236 (21.6)	344 (30.8)	474 (39.7)
AJCC stages			
I	693 (63.3)	575 (51.5)	535 (44.8)
II	316 (28.9)	411 (36.8)	486 (40.7)
III	86 (7.9)	131 (11.7)	174 (14.6)
Grades			
G1	409 (37.4)	249 (22.3)	82 (6.9)
G2	654 (59.7)	784 (70.2)	702 (58.7)
G3	32 (2.9)	84 (7.5)	411 (34.4)
Chemotherapy			
No	954 (87.1)	961 (86.0)	815 (68.2)
Yes	141 (12.9)	156 (14.0)	380 (31.8)

AJCC American Joint Committee on Cancer, *BMI* body mass index

with higher Ki-67 levels received adjuvant or neoadjuvant chemotherapy more often (Table 1).

There were 166, 161, and 259 events in the groups of patients with low, intermediate, and high levels of Ki-67 expression, respectively. The 5-year DFS rates for those groups were 0.90 (95% CI 0.88 to 0.92), 0.89 (95% CI 0.87 to 0.91), and 0.77 (95% CI 0.74 to 0.80). Figure 2 shows Kaplan–Meier curves for these Ki-67 categories.

The 5-year DFS rates for Ki-67 relative to tumor stage and other patient and tumor characteristics are shown in Table 2. Analysis of the subgroups defined by Ki-67 and disease stage (as the strongest prognostic factor) differentiated groups of patients who have a 5-year DFS rate of 0.93 (95% CI 0.91 to 0.95) in patients with stage I tumors and Ki-67 < 10%; and patients with a 5-year DFS rate of 0.49 (95% CI 0.41 to 0.58) in patients with stage III tumors and Ki-67 ≥ 20%. In patients with stage II tumors, Ki-67 separated the patients into groups with a 5-year DFS of 0.86 (95% CI 0.82 to 0.90) in patients with Ki-67 < 10%; 0.90 (95% CI 0.86 to 0.93) in patients with a Ki-67 of 10–19%; and 0.77 (95% CI 0.72 to 0.91) in patients with Ki-67 ≥ 20%. Figure 3 shows Kaplan–Meier curves for the subgroups defined by disease stage and Ki-67.

The effect of Ki-67 on DFS varied in patient groups defined by age (interaction P = 0.01) and grade (interaction P < 0.01). In younger patients (< 45 years), there were survival differences between patients with low Ki-67 expression and those with intermediate Ki-67 expression. This difference was not observed in other age classes (Supplementary Fig. 1). The survival curves for patients with G1, G2, and G3 tumors differed in those with intermediate or high
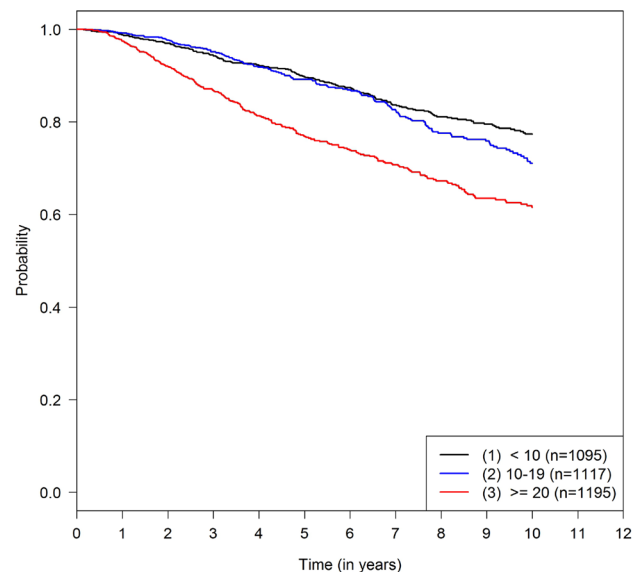
Fig. 2 Disease-free survival relative to Ki-67 categories (corresponding hazard ratios are shown in Table 3)

Table 2 Five-year survival rates (and 95% confidence intervals) relative to patient subgroups and Ki-67 categories

Characteristics	Ki-67 < 10%	Ki-67 10–19%	Ki-67 ≥ 20%
Age at diagnosis (years)			
< 45	0.86 (0.79, 0.94)	0.93 (0.87, 0.99)	0.73 (0.66, 0.81)
45–54	0.95 (0.92, 0.98)	0.92 (0.89, 0.96)	0.76 (0.70, 0.82)
55–64	0.92 (0.89, 0.95)	0.93 (0.90, 0.97)	0.81 (0.76, 0.87)
≥ 65	0.85 (0.82, 0.89)	0.84 (0.80, 0.88)	0.76 (0.71, 0.81)
BMI (kg/m^2)			
< 25	0.89 (0.86, 0.92)	0.89 (0.86, 0.93)	0.76 (0.72, 0.81)
25–30	0.90 (0.87, 0.94)	0.92 (0.89, 0.95)	0.78 (0.73, 0.84)
≥ 30	0.91 (0.87, 0.96)	0.85 (0.79, 0.90)	0.76 (0.70, 0.82)
T			
1	0.93 (0.91, 0.95)	0.93 (0.91, 0.96)	0.86 (0.83, 0.90)
2	0.85 (0.80, 0.89)	0.87 (0.83, 0.91)	0.72 (0.67, 0.77)
3	0.81 (0.67, 0.98)	0.75 (0.62, 0.90)	0.60 (0.48, 0.77)
4	0.68 (0.50, 0.93)	0.66 (0.51, 0.87)	0.49 (0.35, 0.69)
N			
pN0	0.92 (0.90, 0.94)	0.94 (0.92, 0.96)	0.84 (0.81, 0.87)
pN+	0.82 (0.76, 0.87)	0.80 (0.76, 0.85)	0.66 (0.61, 0.72)
AJCC stages			
I	0.93 (0.91, 0.95)	0.94 (0.91, 0.96)	0.87 (0.84, 0.91)
II	0.86 (0.82, 0.90)	0.90 (0.86, 0.93)	0.77 (0.72, 0.81)
III	0.77 (0.68, 0.87)	0.70 (0.61, 0.79)	0.49 (0.41, 0.58)
Grades			
G1	0.91 (0.88, 0.94)	0.98 (0.96, 1.00)	0.89 (0.81, 0.99)
G2	0.89 (0.87, 0.92)	0.88 (0.85, 0.90)	0.78 (0.74, 0.82)
G3	0.82 (0.68, 0.98)	0.83 (0.74, 0.92)	0.73 (0.67, 0.78)
Chemotherapy			
No	0.90 (0.88, 0.92)	0.90 (0.87, 0.92)	0.80 (0.77, 0.84)
Yes	0.88 (0.83, 0.94)	0.86 (0.81, 0.92)	0.69 (0.64, 0.75)

BMI body mass index

Fig. 3 Disease-free survival relative to Ki-67 and disease stage categories

Table 3 Multivariate Cox regression model, showing hazard ratios with 95% confidence intervals and associated *P* value

Characteristics	HR (95% CI)	*P* values
Age (years)		
< 45	1 (Reference)	–
45–54	0.80 (0.59, 1.07)	0.13
55–64	0.81 (0.61, 1.08)	0.15
≥ 65	1.36 (1.05, 1.76)	0.02
BMI		
< 25	1 (Reference)	–
25–29.99	0.79 (0.65, 0.96)	0.02
≥ 30	0.98 (0.79, 1.20)	0.82
AJCC stages		
I	1 (Reference)	–
II	1.76 (1.45, 2.14)	< 0.000001
III	4.61 (3.72, 5.71)	< 0.000001
Ki-67 (%)		
< 10	1 (Reference)	–
10–19	1.03 (0.82, 1.28)	0.82
≥ 20	1.76 (1.44, 2.15)	< 0.000001

AJCC American Joint Committee on Cancer, *BMI* body mass index, *CI* confidence interval, *HR* hazard ratio
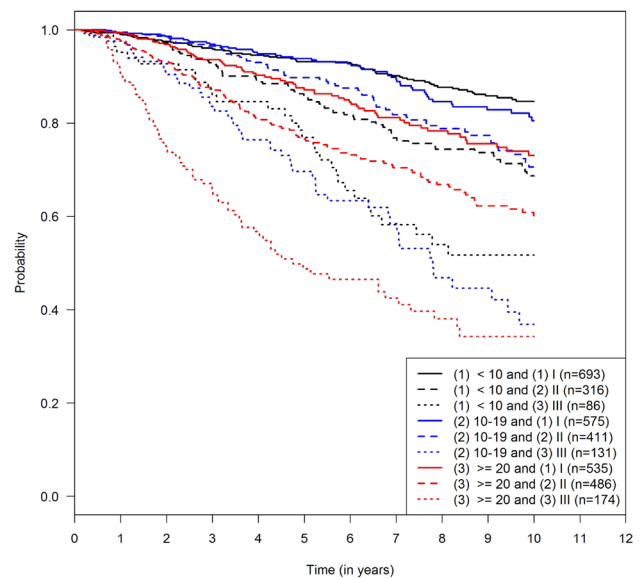
Ki-67 values, but there were hardly any differences between patients with G1 tumors and those with G2 tumors if Ki-67 was low (Supplementary Fig. 5). Different prognostic effects of Ki-67 were not observed in any other patient subgroups.

The Cox regression model, including age, BMI, disease stage, and Ki-67 as predictors of DFS showed that each of these variables was associated with the DFS. HRs are shown in Table 3.

Discussion

This analysis presents 5-year DFS rates for patients with HER2− and hormone receptor-positive early breast cancer in relation to Ki-67 expression and common tumor and patient characteristics. It was found that the prognostic effect of Ki-67 differs in patient subgroups defined by age and grade. In patients with stage I disease, the absolute effects were small. In patients with stage II disease, patients with low and intermediate Ki-67 expression had a similar 5-year DFS, while patients with Ki-67 expression > 20% had a more unfavorable 5-year DFS rate.

Discussions regarding Ki-67 have so far focused on whether it can be used to assist decision-making for or

against adjuvant chemotherapy. Although adjuvant chemotherapy is considered to improve the 10-year overall survival of breast cancer patients by 6–8% [16], this benefit remains questionable in view of the associated toxicity, particularly since new diagnostic methods are now able to identify patients with a prognosis that is favorable enough for chemotherapy to be avoided. Although a few multigene assays have been developed to assist decision-making [6, 37, 38], simpler models have also been established that include Ki-67 expression in the prognostic prediction [12]. In addition, it has been shown in various trials that Ki-67 also has a prognostic effect in patients who do not receive chemotherapy [49]. Another effect of Ki-67 expression that has been described is its value for predicting pCR following neoadjuvant chemotherapy [17, 31, 39]. However, not only because of its limited analytical validity, the clinical utility of Ki-67 expression in decision-making for or against chemotherapy is still under discussion [2, 5, 49]. It has been argued that it may not be able to identify a group of patients who have an excellent prognosis, in whom a decision in favor of chemotherapy is questionable [2].

On the one hand, avoiding chemotherapy in a group of patients with a favorable prognosis is one clinical question. On the other, additional therapy with an acceptable toxicity profile might be beneficial if it was possible to use a relevant marker to identify a group of patients with an unfavorable prognosis. CDK4/6i might be an example of such a treatment. Although it has only been approved for patients with advanced breast cancer, several clinical trials are ongoing to investigate its efficacy in the adjuvant setting [8–10]. Ki-67 expression might be a useful marker for decision-making here, since it is easier to identify women with a poorer prognosis than to identify those with an excellent prognosis. The present study shows that the previously reported cut-off value of 20% identifies a group of patients who have a 5-year DFS of less than 80% in patients with stage II disease. As a matter of fact, this group of patients seems to be a group with yet unmet medical need. Such a patient group might, therefore, benefit from additional therapy like but not restricted to CDK4/6i.

This study has several strengths and limitations. Some of these limitations are associated with Ki-67 staining and assessment and relate to previously identified and discussed concerns regarding the usage of Ki-67 in clinical routine and across institutions [15]. First, with regard to the staining we used MIB-1 as an antibody. MIB-1 is well established for the evaluation of Ki-67 in breast cancer [15]. Therefore, we chose this antibody as a standard for clinical routine assessments. In Germany, the quality of those assessments is validated annually in round robin tests. However, there are further antibodies like SP6. We did not compare Ki-67 assessments with different antibodies which might be a weakness, but other studies showed an extremely high concordance when comparing proliferation assessment with several antibodies [44, 50]. Another issue, which is associated with Ki-67 assessment is the intra-tumor heterogeneity. It is well known that Ki-67 is prone to a relevant intra-tumor heterogeneity [4, 22, 26]. This raises the question how Ki-67 values are assessed to optimize the prediction of prognosis or therapy responsiveness. We used a method, which is also referred to as "hot-spot" method, taking the area into account, which has the highest frequency of positively stained cells. Comparing the hot-spot method versus the average, both methods correlate highly with each other and predict prognosis similarly well [27].

Another concern with regard to Ki-67 and our analysis could be its influence on therapy decisions. While Ki-67 was not used at our site as a standard marker for therapeutic decisions, the physicians attending the tumor board were aware of the Ki-67 value when making those decisions. Therefore, it cannot be excluded that some therapeutic bias was introduced by Ki-67 in our cohort. With regard to the Ki-67 cut-off we pre-defined 10% and 20% as cut-offs for categories, based on clinical studies published with these cut-offs [49]. Furthermore, a cut-off of 20% was considered as being the most clinically relevant cut-off by the St Gallen Expert Panel [25]. However, neither a cut-off of 10% or 20% represent the best separation between intrinsic subtypes [7]. However, utilizing Ki-67 for clinical decision-making, even other cut-offs are discussed like 35% which might be optimal to predict neoadjuvant chemotherapy effectiveness [13, 17]. One strength of the study is clearly the sample size, which made it possible to assess survival rates in subgroups with different levels of Ki-67 expression.

In conclusion, this study shows that Ki-67 expression is able to distinguish between different prognostic groups in most subgroups of patients and tumors. Particularly in patients with AJCC stage II disease, those with Ki-67 expression over 20% may benefit from additional treatments, if available.

Compliance with ethical standards

Ethical approval Approval for this retrospective study was obtained from the Ethics Committee of the Faculty of Medicine at Friedrich Alexander University of Erlangen-Nuremberg. All procedures were in accordance with the Ethical Standards of the Institutional and/or National Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from each individual participant included in the study.

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