Anti-Phosphohistone H3-Positive Mitoses Are Linked to Pathological Response in Neoadjuvantly Treated Breast Cancer

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Keywords

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Summary

Background: We evaluated breast cancer (BC) core biopsies taken before neoadjuvant chemotherapy (NACT) by immunohistochemistry using anti-phosphohistone H3 (PHH3) antibody to determine mitosis, and correlated the results to clinicopathological data and histopathological regression of resected tumor specimens after NACT. **Methods:** 72 patients with either triple-negative (TN) or luminal type BC received NACT with epirubicin/cyclophosphamide (EC) and Taxotere[®]. Tumor regression was analyzed in resected specimens; pathological complete response (pCR) was achieved in 22.2%. Immunohistochemistry with PHH3 was performed on biopsy samples taken before treatment, and mitotic figures were evaluated in 10 high-power fields (HPF). **Results:** PHH3-detected mitoses correlated significantly with tumor grading ($p = 0.001$). TNBC showed > 10 PHH3-positive mitoses/10 HPF significantly more frequently than luminal type BC ($p = 0.003$). Tumors with > 10 PHH3-positive mitoses/10 HPF achieved pCR significantly more often than those with ≤ 10 PHH3-positive mitoses/10 HPF ($p = 0.031$). Even luminal type BC with > 10 PHH3-positive mitoses/10 HPF was associated significantly with pCR compared to luminal type BC with ≤ 10 PHH3-positive mitoses/10 HPF ($p = 0.016$). **Conclusion:** NACT with

EC and Taxotere is suitable for strong proliferating TNBC and luminal BC (> 10 PHH3-positive mitoses/10 HPF). Immunohistochemical determination of mitoses using anti-PHH3 antibody is a simple and robust method for predicting therapy response to NACT in BC tissue.

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Introduction

Patients with locally advanced breast cancer (BC) are treated with neoadjuvant chemotherapy (NACT) to reduce the tumor size before breast-conserving surgery [1]. Adjuvant systemic therapies may also erase micrometastases and enhance overall survival [2, 3]. Pathological complete response (pCR) is generally the primary endpoint in NACT trials, correlates significantly with effective long-term outcome, and was proposed to be a marker for survival [4, 5]. In BC treated with NACT, pCR is one of the most important independent prognosticators for prolonged disease-free survival and overall survival [6, 7]. Therefore, the identification of factors and markers that predict pCR has been the subject of many analyses.

Previous studies identified the number of mitoses evaluated by the mitotic activity index (MAI) as a predictor for the success of NACT [8]. The determination of MAI is a simple and inexpensive method counting well-defined mitotic figures. MAI is usually assessed in hematoxylin and eosin (H&E)-stained tissue specimens by light microscopy at 400-fold magnification in 10 fields of vision

(10 high power fields (HPF)). Nevertheless, in routine pathology, H&E-based MAI quantification represents a time-consuming process with restricted interobserver reproducibility as potential mitotic figures can be interpreted differently by pathologists [9]. By contrast, immunohistochemistry (IHC)-based analyses of mitotic figures (e.g. using anti-phosphohistone H3 (PHH3) antibody) facilitates the interpretation for pathologists. Another well-established method to evaluate the proliferative potential of tumor tissue is the determination of Ki67 by IHC using the MIB-1 antibody. The determination of Ki67 also helps predict response to NACT [10, 11]. Ki67 IHC detects proliferating cells in the G1, S, and G2 phases and during mitosis, but not in the G0 phase [12]. Thus, MIB-1 positivity is not restricted only to mitotic cells, but rather reflects the activity of proliferation in a given tissue.

In this study, by IHC using the recently described anti-PHH3 antibody [13], we analyzed the number of mitoses detected by PHH3 expression in 10 HPF. Histone H3 is a core protein and, as other histones, part of the nucleosome octamer. Its phosphorylation at Ser¹⁰ and Ser²⁸ is exclusively connected to the chromatin condensation during mitosis and meiosis [14]. Interestingly, this phosphorylation process is not observed during apoptosis [15]. Thus, PHH3 staining allows the reliable identification of mitoses and the differentiation between mitotic and apoptotic cells.

We have shown that the number of anti-PHH3-positive mitoses is correlated to different clinicopathological parameters. We also investigated whether the PHH3 mitotic count has a predictive value for the response to NACT with epirubicin/cyclophosphamide (EC) and Taxotere®.

Materials and Methods

Patients

Our study cohort included 72 female BC patients: 60 had invasive ductal adenocarcinomas and 12 invasive lobular carcinomas according to the 2003 WHO criteria [16]. All patients were treated at the Department of Obstetrics and Gynecology, Freiburg University Medical Center, Freiburg, Germany, between 2002 and 2008. The diagnosis was histopathologically confirmed by core needle biopsy of the tumor and included the WHO classification of 2003, the histological grading according to Elston and Ellis [17] and the evaluation of estrogen (ER) and progesterone receptors (PgR) [18] and human epidermal growth factor receptor 2 (HER2neu) by immunohistochemistry [19]. If a score of 2+ was found for HER2neu, chromogenic in situ hybridization was performed. In this study, patients with 2 different intrinsic molecular subtypes were included: 22 with triple-negative BC (TNBC) without any expression of ER, PgR or HER2 (ER-/PgR-/HER2-) and 50 with so-called luminal type BC (ER+ and/or PgR+ and HER2-) according to the St. Gallen guidelines 2011 [20]. After diagnosis, patients received 4 cycles of NACT with EC (90 mg/m² epirubicin and 600 mg/m² cyclophosphamide) and Taxotere (100 mg/m²) followed by surgical treatment with breast-preserving therapy or mastectomy. All histological tissues obtained at operation were sampled and archived at the Institute of Pathology, Freiburg University Medical Center, and were graded, classified, and staged according to the references of the WHO 2003 [16] and the UICC 7th edition [21] by 2 experienced pathologists blinded to the clinical data.

Evaluation of the Response to NACT

After surgical treatment the histopathological response to NACT was assessed according to a routinely applied regression grading system [22]. For this score, H&E-stained tissues of the tumor region after NACT was used. Pre-

treated cancer tissues were examined by light microscopy for regressive changes using a semiquantitative scoring system from 4 to 0. Regression score 4 means that there are no tumor cells (neither invasive cancer, nor in-situ carcinoma cells) detectable, score 3 that there is only residual non-invasive (in-situ) tumor, score 2 that there are only minimal residual invasive tumor foci (≤ 0.5 cm), score 1 that there is invasive cancer with tumor sclerosis and resorption, and score 0 that there is no therapeutic effect. Many studies assess pCR as the absence of invasive carcinoma, but allow the presence of in-situ carcinoma foci because they do not affect the outcome compared to patients without invasive carcinoma [23]. Therefore, in this study pCR is taken to correspond to regression scores 4 and 3.

Immunohistochemistry

Specimens (2 μ m) of all patients tissue samples (formalin-fixed, 10% neutral-buffered and paraffin embedded) were cut for IHC analyses. For PHH3 IHC, all sections were treated simultaneously to ensure a standardized processing of the samples. Sections were mounted onto slides (SuperFrost® Plus slides, Langenbrinck, Emmendingen, Germany) and dried overnight (58°C). After dewaxing in Xylene and rehydrating in decreasing alcohol concentrations, the sections were incubated with TRS (Target retrieval solution, 1:10 dilution, Dako, Glostrup, Denmark) for 20 min (pH 9) and then cooled on ice (10 min). Sections were washed in distilled water and in Dako wash buffer (1:10 dilution) for 10 min each and encircled using a Dako-Pen. Rabbit polyclonal anti-PHH3 (Ser¹⁰) mitosis marker (Millipore no. 06-570; Billerica, MA, USA) was used at a dilution of 1:5,000, diluted with Dako Real™ antibody solution and then incubated with wash buffer (Dako) for 10 min. The immune complex was visualized with the Dako REAL™ Detection System, alkaline/phosphatase/RED, rabbit/mouse (K5005; Dako). Sections were incubated with Dako Link biotinylated secondary antibodies (AB2, Dako) for 30 min followed by washing with wash buffer (10 min) and with streptavidin alkaline phosphatase (AP, Dako, 15 min) and once again with wash buffer (10 min). Finally, an incubation of the sections with chromogen (as described at the Dako REAL™ Detection System) was stopped with distilled water (10 min) and sections were counterstained with hematoxylin. Dehydration in increasing alcohol and xylol followed before mounting.

Immunostaining for the expression of ER, PgR and HER2/neu was performed on serial tissue sections of core needle biopsy samples taken before treatment. The following primary antibodies were used: monoclonal antibody to ER (clone 1D5; Dako; 1:200 dilution; pH 6); monoclonal antibody to PgR (PgR6636; Dako; 1:200 dilution; pH 6), and the polyclonal antibody to the HER2/neu protein (Dako; 1:350 dilution; pH 6). All stainings were performed on a Dako autostainer according to the instructions of the manufacturer. A nuclear reactivity of ER and PgR in $> 1\%$ of the tumor cells was evaluated as a positive reaction [18]. HER2neu overexpression (HER⁺) was defined as an intense and complete membrane staining in more than 30% of the tumor cells [19]. According to their expression of ER, PgR and HER2, all tumors were divided in luminal type BC (ER+ and/or PgR+/HER2-), TNBC (ER-/PgR-/HER2-), luminal HER2 (ER+ and/or PgR+/HER2+) and HER2-enriched (ER-, PgR- and HER2+) according to the St Gallen guidelines of 2011 [20]. In this study, luminal HER2 and HER2-enriched tumors were not included because they receive another therapy, e.g. with a HER2neu receptor antagonist like Herceptin®.

Scoring and Quantification of PHH3-Detected Mitoses

The number of mitoses detected using PHH3 in cancer cells of biopsy specimens taken before treatment was counted in 10 HPF by 2 experienced pathologists (S.T. and P.B.) simultaneously, using a single multi-view light microscope with 1 pathologist directing the object plate. If findings for a slide deviated, the slide was re-evaluated and the results discussed. Although interobserver agreement was good, all results were controlled and re-evaluated. Only PHH3-positive nuclei in the prophase, metaphase, anaphase and the telophase of mitosis were noted (fig. 1). Interphase figures with an irregular granular PHH3 staining were excluded. Signals from malignant epithelial tumor cells in 10 HPF were recorded; those in tumor stromal cells or blood cells were ignored. This evaluation scheme has been described in detail previously [24]. 1 HPF was represented by an areas size of 0.2376 mm² (i.e. 10 HPF = 2.376 mm²).

Statistical Data Analyses

For statistical evaluation the software package SPSS v 18 (SPSS, Inc., Chicago, IL, USA) was used. The parameter PHH3-positive mitoses/10 HPF (2.376 mm²) was found to be normally distributed using the Kolmogorov-Smirnov (K-S) test. Therefore, results were analyzed by the 1-way analysis of variance (ANOVA) for normally distributed data and the 2-sided Fisher's exact test for binary or nominal data. A significance level of 5% was chosen. In addition, we assessed a cut-off value close to the 25th percentile, which is 9.25 PHH3/10 HPF, and set this value up to 10 PHH3-positive mitoses/10 HPF.

Fig. 1. **A** Anti-phosphohistone H3 (PHH3)-stained tissue section of a core needle biopsy specimen (1× magnification). **B** Anti-PHH3-positive mitoses in the prophase (#) and metaphase (*; 40× magnification, red staining). **C** Mitoses in the anaphase (#) and telophase (*; 40× magnification, red staining).

A multivariate analysis was performed to determine the predictive value of PHH3. For relevant parameters determined by univariate analyses ($p \leq 0.1$: intrinsic subtype, grading; $> \text{or} \leq 10$ PHH3-positive mitoses/10 HPF), odds ratio (OR) was calculated for pCR versus no pCR with a logistic regression model.

Results

Pretherapeutic Biopsies

We counted PHH3-immunostained mitoses/10 HPF in breast cancer tissues obtained from 72 patients before NACT. The median value for mitoses detected by PHH3/10 HPF was 30.00 mitoses/10 HPF. Of these, 6/72 cases were well differentiated, 40/72 moderately and 16/72 poorly differentiated carcinomas, and PHH3 expression differed significantly between those 3 histological gradings ($p = 0.001$; fig. 2A). Invasive ductal carcinomas showed more PHH3-positive mitoses (median value 30.00 mitoses/10 HPF) than invasive lobular carcinomas (median value 8 mitoses/10 HPF), but without statistical significance ($p = 0.202$). Most tumors were larger than 2 cm and less than 5 cm before NACT (cT2) with an average of 41.48 PHH3-positive mitoses/10 HPF. The majority were cN1 (median value 29.00 mitoses/10 HPF). No significant correlations between tumor size (cT) and nodal stage (cN) before therapy and PHH3 expression were noted. The cohort contained 21 TNBC and 51 luminal type BC. TNBC displayed significantly more PHH3-detected mitoses/10 HPF compared to luminal type BC ($p = 0.003$; fig. 2B). These results are given in detail in table 1.

Correlations to Pathological Tumor Regression

Of the 72 patients, 16 achieved pCR (regression score 3 or 4). By univariate analyses we found no significant correlations between pCR and the WHO type ($p = 0.722$), the histological grading

Fig. 2. **A** The number of PHH3-positive mitoses/10 high-power fields (HPF; 2.376 mm²) correlated significantly with the histological grading according to Elston/Ellis ($p = 0.001$). **B** Triple-negative breast cancer (TNBC) had significantly more PHH3-positive mitoses/10 HPF compared to luminal type BC ($p = 0.003$). **C** Poorly differentiated (G3) BC showed pathological complete response (pCR) most frequently, while none of the well-differentiated (G1) tumors reached pCR. Statistically, a trend was seen ($p = 0.114$). **D** TNBC patients also reached pCR more often than patients with luminal type BC ($p = 0.012$). Light gray bars: pCR; dark gray bars: no pCR.

Table 1. Clinicopathological parameter before NACT and their correlation to PHH3-immunostained mitoses/10 HPF (n = 72)

	n (%)	PHH3/10 HPF (2.376 mm ²) median (min; max)	p*
WHO type			
invasive ductal	60 (83.3)	40.0 (0; 163)	0.23
invasive lobular	12 (16.7)	8.0 (1; 119)	
Grading			
G1	6 (8.3)	7.5 (1; 16)	0.001
G2	40 (55.5)	19.0 (0; 120)	
G3	26 (36.2)	52.0 (11; 163)	
UICC parameters			
cT1	9 (13.4)	39.0 (1; 70)	> 0.1
cT2	47 (70.1)	41.5 (0; 163)	
cT3	4 (6.0)	56.5 (1; 89)	
cT4	7 (10.5)	8.0 (3; 30)	
missing	5		
cN0	30 (42.9)	36.0 (0; 119)	> 0.1
cN1	36 (51.4)	33.0 (1; 163)	
cN2	3 (4.3)	8.0 (5; 64)	
cN3	1 (1.4)	33.0 (-)	
missing	2		
Intrinsic subtype			
TNBC	21 (29.1)	53.0 (5; 163)	0.003
luminal type BC	51 (70.9)	21.0 (0; 120)	
Regression score ^a			
0	5 (7)	35.3 (5; 70)	0.340
1	40 (55.5)	41.5 (1; 163)	
2	11 (15.3)	20.7 (0,61)	
3	4 (5.5)	34.5 (18; 78)	
4	12 (16.7)	48.4 (8; 107)	
Response			
no pCR	56 (77.8)	39.9 (0; 163)	0.399
pCR	16 (22.2)	44.9 (8; 107)	

^aRegression score according to Sinn et al. [22].

NACT = neoadjuvant chemotherapy, n = number of patients for the analyzed parameter, PHH3 = phosphohistone H3, HPF = high power fields, min = minimum value, max = maximum value, G1 = well differentiated, G2 = moderate differentiated, G3 = poorly differentiated, cT = tumor size before therapy, cN = nodal stage before therapy, BC = breast cancer, TNBC = triple-negative BC, pCR = pathological complete response.

*One-way analysis of variance.

(p = 0.114; fig. 2C), tumor size (cT; p = 0.234) or nodal stage (cN; p = 0.762). Regarding the intrinsic subtype, patients with TNBC gained pCR significantly more often than those with luminal type BC (p = 0.012; fig. 2D; table 2).

The absolute number of PHH3-detected mitoses/10 HPF did not correlate significantly with tumor regression (table 1), the 5 different regression scores (p = 0.340; fig. 3A), or pCR (p = 0.399; fig. 3B). BC with an excellent response and no residual cancer cells (regression score 4) showed the highest mitotic index (mean value 48.42 PHH3-positive mitoses/10 HPF), but BC with poor therapy response (regression score 0 and 1) also revealed elevated PHH3-positive mitoses. By contrast, by defining 10 PHH3-detected mitoses/10 HPF as a cut-off value, we found that tumors (luminal

Fig. 3. PHH3-detected mitoses and their relation to response/pCR. The absolute number of PHH3-positive mitoses did not correlate to response to neoadjuvant chemotherapy (NACT) determined by the regression score according to Sinn et al. [22] (A) or to pCR (B). Interestingly, tumors without invasive residual cancer (score 4) offered the highest average number of mitoses (A). C PHH3 cut-off value and response: Tumors with > 10 PHH3-positive mitoses/10 HPF had a pCR significantly more often than those with ≤ 10 PHH3-positive mitoses/10 HPF (p = 0.031). Light gray bars: cases with ≤ 10 PHH3-positive mitoses/10 HPF; dark gray bars: cases with > 10 PHH3-positive mitoses/10 HPF.

Table 2. Influence of several clinicopathological parameters and PHH3-positive mitoses/10 HPF on response (pCR/no pCR) (n = 72)

Parameter	n	No pCR, n (%)	pCR, n (%)	p*
WHO type				
Invasive ductal	60	47 (78.3)	13 (21.7)	0.722
Invasive lobular	12	9 (75)	3 (25)	
Grading				
G1	6	6 (100)	0 (0)	0.114
G2	40	33 (82.5)	7 (17.5)	
G3	26	17 (65.4)	9 (34.6)	
UICC parameters				
cT1	9	9 (100)	0 (0)	0.234
cT2	47	33 (70.2)	14 (29.8)	
cT3	4	3 (75)	1 (25)	
cT4	7	6 (85.7)	1 (14.3)	
cN0	30	23 (76.7)	7 (23.3)	
cN1	36	29 (80.5)	7 (19.5)	
cN2	3	2 (66.7)	1 (33.3)	0.762
cN3	1	1 (100)	0 (0)	
Intrinsic subtype				
TNBC	21	12 (57.1)	9 (42.9)	0.012
Luminal type BC	51	44 (86.3)	7 (13.7)	
PHH3				
≤ 10/10 HPF	20	19 (95)	1 (5)	0.031
> 10/10 HPF	52	37 (71.2)	15 (28.8)	
Combinations				
TNBC	21	12 (57.1)	9 (42.9)	0.016
Luminal ≤ 10/10 HPF	19	18 (94.7)	1 (5.3)	
Luminal > 10/10 HPF	32	26 (81.3)	6 (18.7)	
TNBC	21	12 (57.1)	9 (42.9)	0.022
Luminal G1/G2	41	36 (87.8)	5 (12.2)	
Luminal G3	10	8 (80)	2 (20)	

pCR = pathological complete response, n = number of patients for the analyzed parameter, G1 = well differentiated, G2 = moderate differentiated, G3 = poorly differentiated, cT = tumor size before therapy, cN = nodal stage before therapy, BC breast cancer, TNBC = triple negative BC, PHH3 = phosphohistone H3, HPF = high power fields.

*Fisher exact test.

type BC as well as TNBC) with > 10 PHH3-positive mitoses/10 HPF achieved pCR significantly more often than those with ≤ 10 PHH3-positive mitoses/10 HPF (p = 0.031; table 2 and fig. 3C).

Since it was of particular interest to analyze the regression potential of luminal type BC, we subclassified luminal type BC according to the cut-off value. We observed significant differences between TNBC, luminal BC with > 10 PHH3-positive mitoses/10 HPF and luminal BC with ≤ 10 PHH3-positive mitoses/10 HPF with respect to response (p = 0.016; table 2). Only 1 of 7 (14.2%) luminal type BC achieving pCR showed ≤ 10 PHH3-positive mitoses/10 HPF, while 6 of 7 (85.8%) luminal type BC reaching pCR had > 10 PHH3-positive mitoses/10 HPF. All 9 TNBC with pCR had > 10 PHH3-positive mitoses/10 HPF.

We also estimated the relationship between histological grading and response, especially for luminal type BC. 80.3% of all luminal

Table 3. Multivariate analysis for the prediction of pCR using a logistic regression model

Parameter	pCR, n (%)	OR	95% CI	p*
Intrinsic subtype				
TNBC	9 (42.9)	1	–	0.10
luminal type BC	7 (13.7)	3.014	0.807–11.257	
PHH3				
≤ 10/10 HPF	1 (5)	1	–	0.21
> 10/10 HPF	15 (28.8)	0.237	0.025–2.248	
Grading				
G1	0 (0)	1	0.000	0.999
G1 vs. G2	7 (17.5)	0.000	0.000	
G1 vs. G3	9 (34.6)	0.000	0.000	0.999

pCR = pathological complete response, n = number of patients for the analyzed parameter, OR = odds ratio, CI = confidence intervals.

*Logistic regression.

type BC were well (G1) or moderately (G2) differentiated; 19.7% were poorly (G3) differentiated. Interestingly, none of the well-differentiated BC achieved pCR (fig. 2C). Comparing TNBC, luminal G1/G2 and luminal G3 carcinomas, we also found significant differences between the 3 groups in relation to pCR (p = 0.022; table 2). However, compared to the histological grading, PHH3 seems to be a stronger discriminator between the 3 groups with respect to response (p = 0.016).

Multivariate Analyses

By univariate analyses we found that tumors with > 10 PHH3/10 HPF had pCR significantly more frequently. We then performed multivariate analyses with PHH3 (cut-off value > 10 PHH3-positive mitoses/10 HPF), the intrinsic subtypes and the grading (table 3). None of the parameters proved to be an independent predictor of pCR, but there was a statistical trend for the intrinsic subtype (TNBC vs. luminal type BC).

Discussion

For NACT-treated BC, the prediction of pCR is a matter of particular interest because pCR is the most important therapeutic endpoint and a good prognosticator for patient outcome [4, 5]. In this study, 22.2% (16/72) of all patients treated by NACT with EC/Taxotere achieved pCR, which is in line with previous observations [25]. NACT with EC/Taxotere contains a taxane (Taxotere®) that inhibits mitoses by binding microtubules [26]. Previous studies have shown that this NACT should be recommended, in particular, for patients with TNBC because this strongly proliferating intrinsic subtype has the best chances reaching pCR using this therapeutic regime [25]. Here, we obtained the best pCR rate for those patients with TNBC (43%; 9/16). All 9 TNBC achieving pCR were highly proliferating tumors with > 10 PHH3-positive mitoses/10 HPF (2.376 mm²). Compared to luminal type BC, TNBC showed a pCR significantly more frequently (fig. 2D).

Independent of the intrinsic subtype, we have shown that BC patients with > 10 PHH3-positive mitoses/10 HPF achieve pCR significantly more often than those with ≤ 10 PHH3-positive mitoses/10 HPF (table 2 and fig. 3C). This result underlines that in strongly proliferating breast cancer (> 10 PHH3-positive mitoses/10 HPF) pCR after NACT with EC/Taxotere is observed more often than in BC with a low proliferation. Correspondingly, these results confirm prior observations [25].

Luminal type BC shows a different outcome and survival in relation to its proliferating behavior. Therefore, we subclassified this group according to level of Ki67 expression (cut-off value 14%) into luminal A (LUMA) and luminal B (LUMB) BC before adjuvant therapy [20]. However, the discrimination of LUMA and LUMB by Ki67 expression is currently not recommended before NACT. Here we were able to show that strongly proliferating luminal type BC with > 10 PHH3-positive mitoses/10 HPF had pCR significantly more often than luminal type BC with ≤ 10 PHH3-positive mitoses/10 HPF. Of the 7 luminal type BC reaching pCR, only 1 displayed ≤ 10 PHH3-positive mitoses/10 HPF.

The investigation of new predictors for the response to NACT is difficult since breast cancer is a (cyto-)genetically heterogeneous disease with many molecular alterations [27]. Thus, detecting many predictors requires extensive and expensive molecular pathological techniques that are often not available in peripheral institutions.

In this study, we examined the ability of the anti-PHH3 antibody to detect mitoses as a potential predictive marker for the response to NACT with EC/Taxotere using IHC, a well-established and easy to perform method. The MAI is generally used to determine the mitotic count. Although this simple and beneficial histopathological method needs only H&E staining, even experienced pathologists can struggle to discriminate between apoptotic cells and mitotic figures. It has been shown previously that the interob-

server agreement on MAI is inferior compared to that seen for PHH3 immunostaining [28, 29]. Another method to determine the growth fraction is IHC using the Ki67 (MIB-1) antibody. Ki67 has been broadly characterized for BC. It is a predictor of the response to chemotherapy [10] and discriminates between LUMA and LUMB with regards to response to adjuvant therapy [30]. However, until now, there have been only few studies referring to this, and no consortial recommendations are given for the use of the Ki67 antibody before NACT [20]. Furthermore, unlike MAI or the anti-PHH3 antibody, Ki67 expression does not reflect the definite number of mitoses, and up to now there have been no evidence-based guidelines for pathologists to perform a reliable Ki67 score in BC [31].

By contrast, the use of PHH3 is a robust, inexpensive and well-established method to detect mitoses by IHC. We found that strongly proliferating breast cancers (TNBC and luminal type BC) with > 10 PHH3-detected mitoses/10 HPF had a higher rate of pCR after NACT with EC and Taxotere. Therefore, the routinely applied detection of mitoses using anti-PHH3 might be reasonable for TNBC and luminal type BC before NACT with EC and Taxotere.

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Disclosure Statement

The authors declare no conflicts of interest.

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