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Review

Clinical validity and clinical utility of Ki67 in early breast cancer

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***Abstract*:** Ki67 represents an immunohistochemical nuclear localized marker that is widely used in surgical pathology. Nuclear immunoreactivity for Ki67 indicates that cells are cycling and are in G1- to S-phase. The percentage of Ki67-positive tumor cells (Ki67 index) therefore provides an estimate of the growth fraction in tumor specimens. In breast cancer (BC), tumor cell proliferation rate is one of the most relevant prognostic markers and Ki67 is consequently helpful in prognostication similar to histological grading and mRNA profiling-based BC risk stratification. In BCs treated with short-term preoperative endocrine therapy, Ki67 dynamics enable distinguishing between endocrine sensitive and resistant tumors. Despite its nearly universal use in pathology laboratories worldwide, no internationally accepted consensus has yet been achieved for some methodological details related to Ki67 immunohistochemistry (IHC). Controversial issues refer to choice of IHC antibody clones, scoring methods, inter-laboratory reproducibility, and the potential value of computer-assisted imaging analysis and/or artificial intelligence for Ki67 assessment. Prospective clinical trials focusing on BC treatment have proven that Ki67, as determined by standardized central pathology assessment, is of clinical validity. Clinical utility has been demonstrated in huge observational studies.

Keywords: breast cancer, endocrine resistance, Ki67, prognosis, therapy response

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Ki67 as an in situ marker for cycling cells

In 1975, Köhler and Milstein[1] published their seminal paper on the production of monoclonal antibodies of predefined specificity by immortalization of murine spleen cells by somatic fusion with a multiple myeloma cell line. Already 5 years later, a group in the institute of pathology in Kiel, Germany, exploited this technique to generate immunohistochemically applicable monoclonal antibodies. By screening the supernatants of the numerous hybridoma clones obtained after immunization of mice with the Hodgkin cell line L428 for their immunohistochemical reaction pattern on tissue sections from tonsils and lymph nodes involved by Hodgkin disease, they identified two interesting antibody producing clones. One reacted with Hodgkin cells and was named Ki-1.[2] The other selectively labeled proliferating cells *in situ* from G1- to M-phase of the cell cycle and received the label Ki-67 according the number of the clone in multi-well plates from which the supernatant was derived.[3] Initially, widespread application of the new immunohistochemical tool in diagnostic practice was prevented by the need of unfixed fresh frozen tissue for Ki67 staining. Applicability to formalin fixed, paraffin-embedded tissue, which represents the vast majority of specimens in pathological archives, was enabled years later by the discovery of Cattoretti and colleagues that microwave heating unmasks the hidden Ki67 antigen.[4]

The cellular function of Ki67 has partly been elucidated. It appears to be a peri-chromosomal chromatin-protein and resides at densely packed regions, probably heterochromatin.[5] The molecular weight of the two proteins which carry the epitopes recognized by the original Ki67 antibody and the MIB antibodies is 395 and 345 kD (MIB stands for the initials of the first author as well as

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the abbreviation of the institution in Borstel, Germany).

The proteins are encoded by two differentially spliced isoforms of mRNA with open reading frames of 9768 and 8688 base pairs. The MIB antibodies were raised against recombinant protein expressed in bacteria. The central part of the mRNA encoding the Ki-67 antigen contains 16 tandemly repeated 366-bp elements, the 'Ki-67 repeats', each including a highly conserved new motif of 66 bp, the 'Ki-67 motif'.[6] The relevant functional quality of the Ki67 protein appears to be constituted by a biological surfactant property to disperse mitotic chromosomes. Therefore, it may play a predominantly biomechanical role in wrapping the mitotic chromosome periphery in a surfactant-like fashion to support intracellular compartmentalization.[7] As a part of the compartmentalization function, the exclusion of mature ribosomes from the nucleus after mitosis depends on Ki-67-regulated chromosome clustering.[8]

Prognostic relevance of Ki67 in breast cancer

As evidenced by gene expression studies, proliferative activity of breast cancer (BC) appears to represent the driving force determining prognosis in BC.[9] As a consequence, a number of methods have been explored to assess the proliferative activity in BC. Some of them, like flow cytometry, are difficult to establish as routine method.[10] Markers, which promised to be applicable as *in situ* detection methods by immunohistochemistry (IHC), like proliferating cell nuclear antigen or topoisomerase II, suffer from the disadvantage that they are not exclusively expressed by proliferating cells but also during DNA damage repair. Peter Hall, in a famous experiment on himself, which included UV light irradiation of the skin on his own forearm followed by repeated punch biopsies and immunohistochemical staining, demonstrated that Ki67 was the only proliferation marker tested, that was expressed selectively in cycling cells but not during DNA repair.[11] Despite the restriction to unfixed frozen tissue Ki67 was soon applied to assess the proliferative activity of BC.[12] The mean value of Ki67 in mammary carcinomas was 16.6%. A comparison of the mean values of Ki-67-positive cells with the histological grade of the tumors showed a correlation between these two variables – that is, histological grade 1 showed 9%, grade 2 16%, and grade 3 26% proliferating cells.[12] A break-through with regard to the potential prognostic relevance of *in situ* proliferation markers in BC applicable by IHC was achieved when formalin-fixed paraffin embedded tissue specimens could be investigated.[13,14] A review of large retrospective studies on BC with extended follow-up revealed that almost all of them could demonstrate prognostic significance of the marker.[15] In these studies with a follow-up periods of at least 5 years, Ki67 besides tumor size, tumor grade, cathepsin-D, S-phase fraction, mitotic index, and vascular invasion showed a significant association with survival outcome measures in patients with early-stage node-negative BC. However, technical difficulties and variations in the measurement remained obstacles to the broad clinical application of all these markers in early BC patients.[15] In addition, prospective clinical data from randomized trials confirming the prognostic relevance were not available until 2016. A large prospective trial on 3198 BC patients could demonstrate the prognostic relevance of Ki67 in univariate analysis besides RNA expression profiling with Oncotype DX recurrence score (RS), nodal status, central and local grade, estrogen receptor (ER) and progesterone receptor (PR), and tumor size.[16] The prognostic relevance of Ki67 was not only demonstrable by central evaluation in the frame of clinical trials.[17] In an attempt to evaluate the routine use and value of Ki-67 as a prognostic marker 'real-world data' from the clinical cancer registry Regensburg (Bavaria, Germany) were analyzed. In 3658 BC cases, Ki67 was routinely assessed in six different institutes of pathology. Independent from the local derivation of the data, a strong correlation was found between grading and Ki-67 ($p < 0.001$). In multivariable analysis including common clinical and histopathological factors, Ki-67 was an independent prognostic parameter both for disease-free survival and for overall survival.[17] There was some variation with regard to the proportion of the different Ki67 categories. The proportion of the low proliferation group ($\leqslant$15%) in the different institutes of pathology ranged from 51% to 62% of cases. With regard to the high proliferating group defined as Ki67 $\geqslant$ 45%, the range was 7% to 12%. The 5-year disease-free survival rate was 86.7% (overall survival 89.3%) in the low proliferating cohort ($\leqslant$15%) and 75.8% (82.8%) in the highly proliferative group, respectively.[17] The approach chosen in this study[17] to discriminate the high from the low proliferating group according to Ki67 with three intermediate groups (16–25%, 26–35%, and 36–45%) differs from other studies

using single stringent cutoff values to define low-risk and high-risk BC, respectively.[18] The 5-year disease-free survival and overall survival were very similar between the 36% and 45% and the >45% group on the one hand and the 16–25%, 26–35%, and ⩽15% group on the other hand, defining a more favorable and an aggressive subgroup. The intermediate groups 16–25% and 26–35% had similar outcomes ranging between the two extremes.[17] In the WSG ADAPT study applying Ki67 and RNA-based gene expression profiles (RS) for prognostic stratification of BC patients, a >95% concordance between high Ki67 > 35% and RS > 25 was observed, leading to an amendment to the study, that high-risk BC could either be defined by RS > 25 or by Ki67 > 35%.[19] In the Plan B study, all patients in the group with Ki-67 ⩾ 40% and PR < 20% had RS > 25.[16] On the ground of Ki67 stained core needle biopsies, the Gepar Trio trial established an identical percentage threshold to define the high risk (35%) besides low and intermediate risk prognostic groups with ⩽15% and 15.1–35%, respectively.[20] The same percentage-defined categorization was also able to predicted complete pathological response to preoperative chemotherapy.[20]

For biomarkers in oncology, the necessity to separate a biological continuum into discontinuous categories of risk stratification to enable application of different therapy regimens provides a well-known and recurring challenge. In this respect, Ki67 does not differ from other attributes of cancer.[21] Clinical decision finding, however, requires reproducible thresholds for standardization of treatment and comparison of outcome data.

According to this requirement, different cutoff levels have been proposed for Ki67. The applied discriminatory thresholds are differing depending on the selection of cases.[22,23] In an attempt to analyze its predictive capacity for the beneficial effect of chemotherapy, Ki67 expression has been retrospectively determined in two randomized trials on adjuvant chemoendocrine therapy in node-negative BC.[22] For this purpose, 2000 tumor cells have been counted.[22] A high Ki-67 labeling index was found to be associated with other factors that predict poor prognosis. Ki-67 labeling index ⩾19% was found to be an independent prognostic factor but it was not predictive of better response to adjuvant chemotherapy in these studies.[22] The results from Breast International Group Trial 1-98 comparing adjuvant tamoxifen with letrozole produced a different cutoff value. Again central evaluation of 2000 tumor cells was performed but the threshold was set here at >11%.[23] In an effort to separate luminal A from luminal B cases, the St. Gallen consensus has proposed a cutoff value of 13.25%,[18,24] which was later abandoned with no further specification of an alternative Ki67 cutoff to distinguish between luminal A and B.[25] From a practical point of view, reproducibility of a pseudo-accurate 13.25% value may be questioned. In addition, the cutoff of 13.25% was derived from tissue-microarrays that did not reflect the properties of BC tissue material in routine diagnostics.[26] In a retrospective study on 1241 patients with Luminal B early stage BC with 1–3 axillary positive nodes who underwent surgery between 1995 and 2005 and received adjuvant hormonotherapy and/or chemotherapy, Ki67 expression identified a subset of patients with Luminal B and node-positive BC who could benefit from addition of adjuvant chemotherapy to endocrine therapy. Dichotomy was observed for Ki67 at 32% level.[27] The cutoff value of 32%[27] might also be pseudo-accurate and most likely provides an over-extension of the information an immunohistochemical marker like Ki67 is able to deliver under routine circumstances. It seems that cutoff levels ranging from 10% to 20% have been the most common to dichitomize populations.[28] However, an expert group was unable to come to consensus regarding the ideal cut point(s) that might be used in clinical practice.[28] In the ongoing monarchE phase III, multicenter, randomized trial in BC patients, with node-positive, luminal human epidermal growth factor receptor 2 (HER2) negative early BC who completed definitive locoregional therapy and are at high risk of disease recurrence on the basis of clinical or pathologic features, Ki-67 score ⩾20% indicated that abemaciclib plus endocrine therapy was beneficial in comparison to endocrine therapy alone.[29] On the basis of this study, KI67, for the first time, became a biomarker with relevance for the choice of therapy because it is an integral part of the corresponding Food and Drug administration approval.[30]

Ki67 as a marker for endocrine responsiveness in BC

Besides its prognostic relevance in BC, Ki67 has been applied to demonstrate sensitivity of luminal BC to endocrine therapy.[31–33] Unlike triple-negative BC or the HER2-type of BC, which may exhibit dramatic response and will even completely vanish in a considerable proportion of

cases during preoperative chemotherapy, luminal cancers usually do not respond with major tumor shrinkage to endocrine therapy, and complete remission is rare. Whereas incomplete remission in response to preoperative therapy in HER2-positive or triple-negative cancers will yield early information on at least partial resistance to therapy in the non-luminal types of BC, resistance to endocrine therapy providing the mainstay of treatment in luminal BC does not become clinically manifest until relapse will have occurred during treatment. As a possible indicator for endocrine sensitivity of luminal BC Ki67 has been proposed. The IMPACT trial determined retrospectively the prognostic impact of a post-therapeutic Ki67 decrease in patients that were treated with preoperative aromatase inhibitors for luminal BC.[31] A higher Ki67 expression after 2 weeks of endocrine therapy was statistically significantly associated with lower recurrence-free survival, whereas higher Ki67 expression at baseline was not. In the 'POETIC trial', 4486 patients were recruited from 2008 to 2014 and randomized to perioperative endocrine therapy or surgery without neoadjuvant therapy.[32] A subgroup with a low baseline Ki67 ($\leqslant$10%) who have a sufficiently good prognosis and do well on standard endocrine therapy alone was identified.[32] The other subgroup with Ki67 $>$ 10% on baseline could be further differentiated by Ki67 response to 2-week preoperative endocrine therapy. The majority converted to a low Ki67 and might not need anything beyond adjuvant endocrine therapy. By contrast, those with a high Ki67 that had remained high after short-term preoperative endocrine therapy should be considered for further adjuvant treatments.[32] A similar approach to guide systemic therapy in early BC by Ki67-determined proliferative response to short-term 3-week preoperative endocrine therapy has been developed in the multicentric, prospective, randomized ADAPT trial.[33] In N0-1 RS12-25 patients with age $<$50 years and treated with endocrine therapy alone, outcome of endocrine responders ($\leqslant$10% Ki67 after 3 weeks) was superior when compared to non-responders.[33] Thus, omission of chemotherapy in early BC of premenopausal patients with limited nodal burden and intermediate RS can be based on an easy accessible prognostic marker, provided by Ki67 response to short-term endocrine therapy.[33] Missing Ki67 response to short-term endocrine preoperative therapy was associated with genetic aberrations, potentially conferring endocrine resistance like TP53 mutation,[34] which has also been found in more than 25% of relapsing luminal BCs under therapy.[35]

High proliferative activity in BRCA-mutated BC

BCs with a germline background of BRCA1 or BRCA2 mutation share some histopathological features like triple negativity but there is no pathognomonic phenotype. One attribute, which BRCA1 germ line mutated BC have in common is the very high Ki67 labeling index usually exceeding 60%. This is also true for BRCA germline-mutated cases with hormone receptor expression. In a retrospective study on BRCA1-associated tumors, all were of high grade, invasive-ductal subtype, and PR and Her2 negative, and 91% of the tumors were negative for ER; 60% of the tumors showed a high expression of Ki67. There was a significant difference with respect to grading ($p = 0.001$ for G3), ER negativity ($p = 0.0075$), Ki67 $>$ 65% ($p = 0.0039$), and triple negativity ($p = 0.0019$) between tumors from mutation carriers and non-carriers.[36]

Methodological controversies regarding Ki67

Assessment of tumor cell growth fraction by *in situ* assessment of a strictly cell-cycle-associated protein appears to provide a rapid and reliable method suitable for routine application in patient care. Although proliferative activity of BC represents a continuously and gradually increasing biological risk factor according to accumulated genetic aberrations and their composition, the clinical need to set categories for selection of treatment options in a dichotomizing 'yes' or 'no' fashion requires the definition of thresholds and demands reproducibility among pathological laboratories. As a matter of fact, microscopic methods involving human judgment are prone for subjectivity and hence limited reproducibility. It has to be kept in mind that this is also the case for the modified Bloom–Scarff–Richardson grading of BC being in use worldwide.[37,38] By counting of mitotic figures, traditional grading also includes proliferative activity, but the reproducibility of counting mitotic figures is poor to moderate.[37] Generalized kappa values indicated substantial agreement for tubule formation (0.64), but only moderate agreement for mitotic count (0.52).[37] Mitotic figures as a microscopic equivalent of proliferative activity in BC pose a number of methodological problems. They have to be

discriminated from apoptotic figures, what may be impossible occasionally. Compared to G1-M-phase detected by Ki-67, the M-phase of the cell cycle is rather short. Therefore, in cases with limited tumor tissue, only few mitotic figures may be encountered leading to a systematic underestimation of grade in core needle biopsies.[39] Another relevant bias of mitotic counts for grading is provided by the variation in size of microscopic fields to which the number of mitotic figures is related.[40] In addition, there is a massive inter-individual variation of tumor cell density in BC specimens from different patients. Consequently, the Ki67 assessed tumor cell growth fraction related to all tumor cells could provide a valuable alternative method to determine proliferative activity in BC. In a large prospective study, it could be shown that modified Bloom–Scarff–Richardson grading by including Ki67 indices instead of mitotic figures yields a significant prognostic variable, even in multivariate analysis, independent from the prognostic information provided by RNA profiling-based RS.[16] Replacement of mitotic figures in the grading scheme was achieved in this study by defining Ki67 index $<15\%$ as low with one scoring point, 15% to $<25\%$ as intermediate with two scoring points and $\geqslant 25\%$ with three scoring points, respectively.[16]

Due to variation in expression of the Ki67 protein during the cell cycle with a maximum in the M-phase, a spectrum of labeling intensities are observable in a given tumor. It has been demonstrated that variation in staining intensities may be responsible for discordant labeling indices when evaluation was performed by different observers.[41] The lowest rate of discordance can be achieved, when every labeling intensity, even weak staining, is counted as positive.[42]

Another matter of controversy regarding Ki67 as biomarker in BC concerns the handling of hotspots. Hotspots represent areas of higher Ki67 labeling index than present in the remaining tumor. Tumor cell heterogeneity, paracrine effects of accompanying non-tumor stromal cells or differences in local supply with nutrients may be responsible for the phenomenon of non-evenly distributed proliferative activity in BC. The dispute goes on whether these areas should be preferentially counted, neglected, or counted in a non-selective manner.[28,42] Because no consented definition of the criteria, which constitute a hotspot does exist the published prospective studies on prognostic significance of Ki67 in BC have included hotspots in a non-selective manner.[16] Up to now, it is not consented how many cells are at least required to form a hotspot and what range of difference in Ki67 index compared to the rest of the tumor is at least necessary to define a hotspot. The International Ki67 in Breast Cancer Working Group has announced that a working party has been established to assess whether overall staining index or peak labeling indices in hotspots is more robust.[28] In a most recent publication, the group stated that the issue of average value across slide *versus* value in hotspot is still controversial.[42] To overcome the difficulties associated with intratumoral proliferative heterogeneity, a scoring app has been devised that guides manual single cell counting to balance the global Ki67 index over areas with high and low proliferation (weighted global score).[42] However, it has been admitted that this scoring method is 'arguably tedious' and is 'not the only scoring system' that achieves analytical validity.[42] Global (average) scores across the section had higher reproducibility than hotspot methods, although differences were not statistically significant.[42] In the tumor center with presumably reduced nutrient and oxygen supply and common fibrotic regression, the proliferation rate tends to be lower in comparison to the invasion front which, therefore appears to be the preferred area for Ki67 assessment.[22,23,43] Heterogeneity of Ki67 staining in BC has even been demonstrated to provide an additional prognostic factor.[44]

Another controversy on the application of Ki67 as biomarker in BC concerns the mode of microscopic evaluation. Already in their first study describing Ki67 as a prognostic marker in BC, Gerdes and co-workers emphasized that the counting of mitotic figures in routinely stained paraffin sections is difficult and time-consuming.[12] Immunohistological labeling with monoclonal antibody Ki-67 by contrast was considered as simple, well within the scope of routine surgical pathology laboratories, and a more objective aid for assessing the grade of malignancy.[12] As stated, tedious counting is hardly compatible with routine microscopic evaluation by pathologists. An evaluation technique labeled as 'eyeballing' appears to be more appropriate for routine pathology. A rough estimate instead of exact counting has already become standard in some areas of predictive pathology, like assessment of programmed death-ligand 1 expression in cancer and has been accepted by oncologists.[45,46] For exact counting of Ki67 index 200–2000 cells have been

evaluated.[22,23,28] Usually, the minimum is set at 200 cells to be counted.[28] In comparison to counting, eyeballing at the higher and lower Ki67 levels, the correlation between the methods of assessment was found to be acceptable with lower concordance in the intermediate cases with 10–25% Ki67 labeling index.[47] In the plan B trial, encompassing 3198 patients the quantitative and semi-quantitative way of Ki67 assessment were compared.[16] Equal prognostic effects of both methods could be demonstrated.[16] This study, like others, before avoided pseudo-accuracy with regard to Ki67 labeling by only discriminating 5% groups (1–5%, 6–10%, 11–15%, and so on).[16,22] Reproduction of thresholds, in particular when they are within the intermediate range of Ki67 labeling index from >10% to 25% may require exact counting. It is in this medium range of Ki67 expression where reproducibility among different laboratories is worst.[47,48]

To overcome the obstacles to reproducibility between different observers and to enable assessment of a higher proportion of tumor cells computer-assisted image analysis (CAIS) has been suggested for evaluation Ki67 staining. Most of the commercially available applications require the definition of a region of interest where the analysis is conducted.[43,49] This implies that subjectivity of observers is not completely eliminated by these devices. We could show that the region-of-interest size impacts on Ki67 quantification by CAIS in BC.[50] Rimm *et al.* have recently compared the performance of automated Ki67 quantification by 10 different software systems combined with seven different slide scanners on a set 30 BC cases. Automated Ki67 assessment showed a between-system agreement that was not superior but only comparable with the inter-observer agreement achieved by standardized pathologist-based Ki67 evaluation.[51]

Quality assurance trials for Ki67

Analytical validity of Ki67 IHC requires careful attention to preanalytical issues and calibrated standardized visual scoring. Participation in and evaluation of quality assurance and quality control programs are recommended to maintain analytical validity.[42] Quality assurance trials have to cover two levels, interlaboratory and interobserver reproducibility, respectively. Interlaboratory concordance can be assessed by immunostaining of identical materials by different laboratories. The obstacle of limited material in the case of BC tissue has been overcome by the use of tissue micro-arrays, which have been applied in quality assurance trials on Ki67, starting in 2002.[48] For the selection of adequate tissue material and appropriate cases, organizers of round-robin tests have to analyze the tissues before distribution among participants of quality assurance trials and they have to predefine the expected correct results.[52] To discriminate interlaboratory from interobserver variation, it is necessary to evaluate the participants staining results. Interlaboratory variation may be caused by differences in the analytical procedure which therefore have to be communicated by participants. Significant differences in the Ki67 labeling indices were observed between different antibody clones (SP6, Ab30.9, MIB1, MM1) and between different stainer platforms (Dako Autostainer, Ventana Bench Mark, Leica Bond).[53] Even the combination of specific platforms with certain antibodies had impact on the Ki67 index, indicating limited reproducibility of too narrowly defined thresholds.[53] Significant variations in the proportion of tumors with Ki67 high-level expression (Ki67 PI$\geqslant$20%) were observed among Ab, format, and stainer platform combinations. In the annually organized Ki67 round-robin tests in Germany, since 2002 staining quality of laboratories has gradually improved.[52] A proportion of discordances is due to interobserver variation as evidenced by central evaluation of all participant's staining results.[52] Although it could be demonstrated that regular participation in Ki67 quality assurance trials has significantly improved the performance of participating laboratories and pathologists,[52] interobserver variance remains a challenge. The latter can only be overcome by training. For this purpose, the quality assurance organization QuIP has set up virtual microscopy of Ki67-stained slides (https://www.qualityinpathology.com/en_GB/zerpa/). Regular participation in quality assurance trials on Ki67 to guarantee analytical validity provides a mandatory prerequisite for the clinical utility of the biomarker Ki67. In recent decades, ample of evidence for the clinical validity of Ki67 has been accumulated, which has to be clearly discriminated from clinical utility.[54] Clinical utility requires reproducibility under routine circumstances, which appears achievable in a trained and standardized environment.[52]

Conclusions

In situ assessment of tumor cell growth fraction by immunhistochemical detection of the strictly proliferation-associated nuclear Ki67 antigen

provides a biomarker with analytical and clinical validity as well as clinical utility in BC[54]:

- Prognostic significance of proliferative activity in BC has long been established, but unlike mitotic figures, which are conventionally used in pathology, Ki67 covers a broader spectrum of the cell cycle, not only M-phase but also the whole cycle from G1 to M-phase and thus can be applied on small tumor cell numbers as in core needle biopsies. Furthermore, tedious counting requested for mitotic figures is not necessary and karyorhectic figures do not pose a discriminatory problem.
- Currently, no other predictive biomarker is available to indicate endocrine resistance or sensitivity in BC which can be studied by Ki67 response to short-term preoperative endocrine therapy of luminal BC. A drop of Ki67 index after short-term preoperative endocrine therapy to ⩽10% is associated with endocrine sensitivity sufficient for growth suppression of tumor cells not requiring chemotherapy for effective long-term relapse-free tumor control.
- No generally accepted Ki67 cutoff value to discriminate prognostic favorable from aggressive BC does exist. But, prospective clinical trials with central pathology as well as decentral observational studies indicate that the risk in BC cases with ⩽10% Ki67 labeling index is minimal, whereas it is very high when ⩾35% is exceeded. At the extreme sides of the spectrum, ⩽10% and ⩾35%, respectively, the reproducibility of Ki67 index has been shown to be much better than in the intermediate range.
- Reproducibility of Ki67-assessed proliferative activity generally does not provide a problem in cases with either low ⩽10% or very high Ki67-determined growth fraction ⩾35%. Mainly for the in-between cases quality assurance trials for interlaboratory concordance of staining and microscopic assessment have been conducted. It could be demonstrated that performance status of laboratories significantly improves with regular participation.
- Bloom–Scarff–Richardson grading of BC according to WHO recommendations has limited reproducibility in a decentral setting.[38] This deficit is partly due to poor reproducibility of mitotic figures.[37] Grading can be improved by replacing mitotic figures by Ki67 staining with retained scoring of tubule formation and nuclear pleomorphism. Thus, modified grading has shown prognostic significance in a huge prospective study on more than 3000 BC patients.[16]

Declarations

Ethics approval and consent to participate
Not applicable.

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Not applicable.

Author contribution(s)
Hans Kreipe: Conceptualization; Writing – original draft; Writing – review & editing.

Nadia Harbeck: Conceptualization; Methodology; Supervision; Writing – review & editing.

Matthias Christgen: Conceptualization; Methodology; Project administration; Validation; Writing – review & editing.

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