Clinical validity and clinical utility of Ki67 in early breast cancer

Hans Kreipe, Nadia Harbeck and Matthias Christgen

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

Ki67 as an in situ marker for cycling cells
In 1975, Köhler and Milstein 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. 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. 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.

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. 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...
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’. 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. As a part of the compartmentalization function, the exclusion of mature ribosomes from the nucleus after mitosis depends on Ki-67-regulated chromosome clustering.

**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. 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. 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. Despite the restriction to unfixed frozen tissue Ki67 was soon applied to assess the proliferative activity of BC. 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. 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. 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. 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. 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. The prognostic relevance of Ki67 was not only demonstrable by central evaluation in the frame of clinical trials. 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. There was some variation with regard to the proportion of the different Ki67 categories. The proportion of the low proliferation group (≤15%) in the different institutes of pathology ranged from 51% to 62% of cases. With regard to the high proliferating group defined as Ki67 ≥ 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 (≤15%) and 75.8% (82.8%) in the highly proliferative group, respectively. The approach chosen in this study 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.
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. It is an integral part of the corresponding Food and Drug administration approval. 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 patients, the estrogen receptor (ER) and progesterone receptor (PR) expression in combination with Ki67 expression may be considered as a potential marker for the selection of patients who will benefit from adjuvant endocrine therapy alone or in combination with chemotherapy. In a recent study, the Ki67 expression was found to be associated with better response to adjuvant endocrine therapy. The Ki67 expression was also found to be associated with improved overall survival in patients who received endocrine therapy alone or in combination with chemotherapy. This finding supports the use of Ki67 as a biomarker for the selection of patients who will benefit from adjuvant endocrine therapy. The Ki67 expression was found to be associated with better response to adjuvant endocrine therapy in patients with hormone-sensitive BC. However, the use of Ki67 as a biomarker for the selection of patients who will benefit from adjuvant endocrine therapy is still limited due to the lack of standardized cutoff values for Ki67 expression. Further research is needed to establish standardized cutoff values for Ki67 expression and to validate the use of Ki67 as a biomarker for the selection of patients who will benefit from adjuvant endocrine therapy.
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. 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. A subgroup with a low baseline Ki67 (\(\leq 10\%\)) who have a sufficiently good prognosis and do well on standard endocrine therapy alone was identified. 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. 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. In N0-1 RS12-25 patients with age < 50 years and treated with endocrine therapy alone, outcome of endocrine responders (\(\leq 10\%\) Ki67 after 3 weeks) was superior when compared to non-responders. 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. Missing Ki67 response to short-term endocrine preoperative therapy was associated with genetic aberrations, potentially conferring endocrine resistance like TP53 mutation, which has also been found in more than 25% of relapsing luminal BCs under therapy.

**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.

**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. By counting of mitotic figures, traditional grading also includes proliferative activity, but the reproducibility of counting mitotic figures is poor to moderate. Generalized kappa values indicated substantial agreement for tubule formation (0.64), but only moderate agreement for mitotic count (0.52). 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. 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. 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. 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 ≥25% with three scoring points, respectively.

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. The lowest rate of discordance can be achieved, when every labeling intensity, even weak staining, is counted as positive. 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. 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. 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. For exact counting of Ki67 index 200–2000 cells have been
evaluated.\textsuperscript{22,23,28} Usually, the minimum is set at 200 cells to be counted.\textsuperscript{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.\textsuperscript{47} In the plan B trial, encompassing 3198 patients the quantitative and semi-quantitative way of Ki67 assessment were compared.\textsuperscript{16} Equal prognostic effects of both methods could be demonstrated.\textsuperscript{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).\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{50} Rimm \textit{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 interobserver agreement achieved by standardized pathologist-based Ki67 evaluation.\textsuperscript{51}

\textbf{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.\textsuperscript{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.\textsuperscript{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 define the expected correct results.\textsuperscript{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).\textsuperscript{53} Even the combination of specific platforms with certain antibodies had impact on the Ki67 index, indicating limited reproducibility of too narrowly defined thresholds.\textsuperscript{53} Significant variations in the proportion of tumors with Ki67 high-level expression (Ki67 PI \textgtrless 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.\textsuperscript{52} A proportion of discordances is due to interobserver variation as evidenced by central evaluation of all participant’s staining results.\textsuperscript{52} Although it could be demonstrated that regular participation in Ki67 quality assurance trials has significantly improved the performance of participating laboratories and pathologists,\textsuperscript{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.\textsuperscript{54} Clinical utility requires reproducibility under routine circumstances, which appears achievable in a trained and standardized environment.\textsuperscript{52}

\textbf{Conclusions}

\textit{In situ} assessment of tumor cell growth fraction by immunohistochemical detection of the strictly proliferation-associated nuclear Ki67 antigen...
provides a biomarker with analytical and clinical validity as well as clinical utility in BC:

- 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 karyorrhectic 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 \( \leq 10\% \) 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 decentralized observational studies indicate that the risk in BC cases with \( \leq 10\% \) Ki67 labeling index is minimal, whereas it is very high when \( \geq 35\% \) is exceeded. At the extreme sides of the spectrum, \( \leq 10\% \) and \( \geq 35\% \), 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 \( \leq 10\% \) or very high Ki67-determined growth fraction \( \geq 35\% \). 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. This deficit is partly due to poor reproducibility of mitotic figures. 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.

**Declarations**

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
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.

**Acknowledgements**
None.

**Funding**
The authors received no financial support for the research, authorship, and/or publication of this article.

**Competing interests**
The authors declare that there is no conflict of interest.

**Availability of data and materials**
Not applicable.

**ORCID iD**
Nadia Harbeck https://orcid.org/0000-0002-9744-7372

**References**
1. Köhler G and Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975; 256: 495–497.
2. Schwab U, Stein H, Gerdes J, et al. Production of a monoclonal antibody specific for Hodgkin and Sternberg-Reed cells of Hodgkin’s disease and a subset of normal lymphoid cells. *Nature* 1982; 299: 65–67.
3. Gerdes J, Lemke H, Baisch H, et al. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. *J Immunol* 1984; 133: 1710–1715.

4. Cattoretti G, Becker MH, Key G, et al. Monoclonal antibodies against recombinant parts of the Ki-67 antigen (MIB 1 and MIB 3) detect proliferating cells in microwave-processed formalin-fixed paraffin sections. *J Pathol* 1992; 168: 357–363.

5. Kreitz S, Fackelmayer FO, Gerdes J, et al. The proliferation-specific human Ki-67 protein is a constituent of compact chromatin. *Exp Cell Res* 2000; 261: 284–292.

6. Schlüter C, Duchrow M, Wohlenberg C, et al. The cell proliferation-associated antigen of antibody Ki-67: a very large, ubiquitous nuclear protein with numerous repeated elements, representing a new kind of cell cycle-maintaining proteins. *J Cell Biol* 1993; 123: 513–522.

7. Cuylen S, Blaukopf C, Politi AZ, et al. Ki-67 acts as a biological surfactant to disperse mitotic chromosomes. *Nature* 2016; 535: 308–312.

8. Cuylen Haering S, Petrovic M, Hernandez-Armendariz A, et al. Chromosome clustering by Ki-67 excludes cytoplasm during nuclear assembly. *Nature* 2020; 587: 285–290.

9. Wirapati P, Sotiriou C, Kunkel S, et al. Meta-analysis of gene expression profiles in breast cancer: toward a unified understanding of breast cancer subtyping and prognosis signatures. *Breast Cancer Res* 2008; 10: R65.

10. Camplenjoh RS, Brock A, Barnes DM, et al. Ki-61, a novel proliferative marker: flow cytometric assessment of staining in human breast carcinoma cells. *Br J Cancer* 1993; 67: 657–662.

11. Hall PA, McKee PH, Menage HD, et al. High levels of p53 protein in UV-irradiated normal human skin. *Oncogene* 1993; 8: 203–207.

12. Gerdes J, Lelle RJ, Pickartz H, et al. Growth fractions in breast cancers determined in situ with monoclonal antibody Ki-67. *J Clin Pathol* 1986; 39: 977–980.

13. Kreipe H, Alm P, Olsson H, et al. Prognostic significance of a formalin-resistant nuclear proliferation antigen in mammary carcinomas as determined by the monoclonal antibody Ki-S1. *Am J Pathol* 1993; 142: 651–657.

14. Sampson SA, Kreipe H, Gillett CE, et al. KiS1—a novel monoclonal antibody which recognizes proliferating cells: evaluation of its relationship to prognosis in mammary carcinoma. *J Pathol* 1992; 168: 179–185.

15. Mirza AN, Mirza NQ, Vlastos G, et al. Prognostic factors in node-negative breast cancer: a review of studies with sample size more than 200 and follow-up more than 5 years. *Ann Surg* 2002; 235: 10–26.

16. Gluz O, Nitz UA, Christgen M, et al. West German Study Group Phase III PlanB Trial: first prospective outcome data for the 21-gene recurrence score assay and concordance of prognostic markers by central and local pathology assessment. *J Clin Oncol* 2016; 34: 2341–2349.

17. Inwald EC, Klinikhammer-Schalke M, Hofstädter F, et al. Ki-67 is a prognostic parameter in breast cancer patients: results of a large population-based cohort of a cancer registry. *Breast Cancer Res Treat* 2013; 139: 539–552.

18. Metzger-Filho O, Sun Z, Viale G, et al. Patterns of recurrence and outcome according to breast cancer subtypes in lymph node-negative disease: results from international breast cancer study group trials VIII and IX. *J Clin Oncol* 2013; 31: 3083–3090.

19. Nitz U, Gluz O, Kreipe HH, et al. The run-in phase of the prospective WSG-ADAPT HR+/HER2- trial demonstrates the feasibility of a study design combining static and dynamic biomarker assessments for individualized therapy in early breast cancer. *Ther Adv Med Oncol* 2020; 12: 1758835920973130.

20. Denkert C, Loibl S, Müller BM, et al. Ki67 levels as predictive and prognostic parameters in pretherapeutic breast cancer core biopsies: a translational investigation in the neoadjuvant GeparTrio trial. *Ann Oncol* 2013; 24: 2786–2793.

21. Denkert C, Budczies J, von Minckwitz G, et al. Strategies for developing Ki67 as a useful biomarker in breast cancer. *Breast* 2015; 24 Suppl 2: S67–S72.

22. Viale G, Regan MM, Mastropasqua MG, et al.; International Breast Cancer Study Group. Predictive value of tumor Ki-67 expression in two randomized trials of adjuvant chemoendocrine therapy for node-negative breast cancer. *J Natl Cancer Inst* 2008; 100: 207–212.

23. Viale G, Giobbie-Hurder A, Regan MM, et al.; Breast International Group Trial 1-98. Prognostic and predictive value of centrally reviewed Ki-67 labeling index in postmenopausal women with endocrine-responsive breast cancer: results from Breast International Group Trial 1-98 comparing adjuvant tamoxifen with letrozole. *J Clin Oncol* 2008; 26: 5569–5575.

24. Goldhirsch A, Winer EP, Coates AS, et al.; Panel members. Personalizing the treatment of women with early breast cancer: highlights of the St
Gallin International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. *Ann Oncol* 2013; 24: 2206–2223.

25. Coates AS, Winer EP, Goldhirsch A, et al.; Panel Members. Tailoring therapies—improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015. *Ann Oncol* 2015; 26: 1533–1546.

26. Cheang MC, Chia SK, Voduc D, et al. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. *J Natl Cancer Inst* 2009; 101: 736–750.

27. Criscitiello C, Disalvatore D, De Laurentiis M, et al. High Ki-67 score is indicative of a greater benefit from adjuvant chemotherapy when added to endocrine therapy in luminal B HER2 negative and node-positive breast cancer. *Breast* 2014; 23: 69–75.

28. Dowsett M, Nielsen TO, A'Hern R, et al.; International Ki67 in Breast Cancer Working Group. Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer Working group. *J Natl Cancer Inst* 2011; 103: 1656–1664.

29. Harbeck N, Rastogi P, Martin M, et al.; monachiE Committee Members. Adjuvant abemaciclib combined with endocrine therapy for high-risk early breast cancer: updated efficacy and Ki-67 analysis from the monarchE study. *Ann Oncol* 2021; 32: 1571–1581.

30. U.S. Food and Drug Administration. FDA approves abemaciclib with endocrine therapy for early breast cancer, www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-abemaciclib-endocrine-therapy-early-breast-cancer (2022)

31. Dowsett M, Smith IE, Ebbs SR, et al.; IMPACT Trialists Group. Prognostic value of Ki67 expression after short-term presurgical endocrine therapy for primary breast cancer. *J Natl Cancer Inst* 2007; 99: 167–170.

32. Smith I, Robertson J, Kilburn L, et al. Long-term outcome and prognostic value of Ki67 after perioperative endocrine therapy in postmenopausal women with hormone-sensitive early breast cancer (POETIC): an open-label, multicentre, parallel-group, randomised, phase 3 trial. *Lancet Oncol* 2020; 21: 1443–1454.

33. Nitz U, Gluz O, Kümmel S, et al.; West German Study Group. Endocrine therapy response and 21-gene expression assay for therapy guidance in HR+/HER2– early breast cancer. *J Clin Oncol* 2022; 40: 2557–2567.

34. Grote I, Bartels S, Kandt L, et al. TP53 mutations are associated with primary endocrine resistance in luminal early breast cancer. *Cancer Med* 2021; 10: 8581–8594.

35. Razavi P, Chang MT, Xu G, et al. The genomic landscape of endocrine-resistant advanced breast cancers. *Cancer Cell* 2018; 34: 427–438.

36. Gadzicki D, Schubert A, Fischer C, et al. Histopathological criteria and selection algorithms for BRCA1 genetic testing. *Cancer Genet Cytogenet* 2009; 189: 105–111.

37. Frierson HF Jr, Wolber RA, Berean KW, et al. Interobserver reproducibility of the Nottingham modification of the Bloom and Richardson histologic grading scheme for infiltrating ductal carcinoma. *Am J Clin Pathol* 1995; 103: 195–198.

38. van Dooijeweert C, van Diest PJ, Willems SM, et al. Significant inter- and intra-laboratory variation in grading of ductal carcinoma in situ of the breast: a nationwide study of 4901 patients in the Netherlands. *Breast Cancer Res Treat* 2019; 174: 479–488.

39. O’Shea AM, Rakha EA, Hodi Z, et al. Histological grade of invasive carcinoma of the breast assessed on needle core biopsy - modifications to mitotic count assessment to improve agreement with surgical specimens. *Histopathology* 2011; 59: 543–548.

40. Ellis O, Simpson JF, Reis-Filho JS, et al. Grading. In: Lakhani SR, Ellis IO, Schnitt SJ, et al. (eds) *WHO classification of tumours of the breast*. Lyon: IARC, 2012, pp.19–20.

41. Varga Z, Diebold J, Dommann-Scherrer C, et al. How reliable is Ki-67 immunohistochemistry in grade 2 breast carcinomas? A QA study of the Swiss Working Group of breast- and gynecopathologists. *PLoS One* 2012; 7: e37379.

42. Nielsen TO, Leung SCY, Rimm DL, et al. Assessment of Ki67 in breast cancer: updated recommendations from the International Ki67 in Breast Cancer Working Group. *J Natl Cancer Inst* 2021; 113: 808–819.

43. Laurinavicius A, Plancoulaine B, Laurinaviciene A, et al. A methodology to ensure and improve agreement with surgical specimens. *Histopathology* 2011; 59: 543–548.

44. Laurinavicius A, Plancoulaine B, Rasmusson A, et al. Bimodality of intratumor Ki67 expression is an independent prognostic factor of overall survival in patients with invasive breast carcinoma. *Virchows Arch* 2016; 468: 493–502.
45. Chebib I and Mino-Kenudson M. PD-L1 immunohistochemistry: clones, cutoffs, and controversies. *APMIS* 2022; 130: 295–313.

46. Hwang DM, Albaqr T, Santiago RC, *et al*. Prevalence and heterogeneity of PD-L1 expression by 22C3 assay in routine population-based and reflexive clinical testing in lung cancer. *J Thorac Oncol* 2021; 16: 1490–1500.

47. van den Berg EJ, Duarte R, Dickens C, *et al*. Ki67 immunohistochemistry quantification in breast carcinoma: a comparison of visual estimation, counting, and immunoratio. *Appl Immunohistochem Mol Morphol* 2021; 29: 105–111.

48. Mengel M, von Wasielewski R, Wiese B, *et al*. Inter-laboratory and inter-observer reproducibility of immunohistochemical assessment of the Ki-67 labelling index in a large multi-centre trial. *J Pathol* 2002; 198: 292–299.

49. Alataki A, Zabaglo L, Tovey H, *et al*. A simple digital image analysis system for automated Ki67 assessment in primary breast cancer. *Histopathology* 2021; 79: 200–209.

50. Christgen M, von Ahsen S, Christgen H, *et al*. The region-of-interest size impacts on Ki67 quantification by computer-assisted image analysis in breast cancer. *Hum Pathol* 2015; 46: 1341–1349.

51. Rimm DL, Leung SCY, McShane LM, *et al*. An international multicenter study to evaluate reproducibility of automated scoring for assessment of Ki67 in breast cancer. *Mod Pathol* 2019; 32: 59–69.

52. Raap M, Ließem S, Rüschoff J, *et al*. Quality assurance trials for Ki67 assessment in pathology. *Virchows Arch* 2017; 471: 501–508.

53. Røge R, Nielsen S, Riber-Hansen R, *et al*. Impact of primary antibody clone, format, and stainer platform on Ki67 proliferation indices in breast carcinomas. *Appl Immunohistochem Mol Morphol* 2019; 27: 732–739.

54. Hayes DF. Defining clinical utility of tumor biomarker tests: a clinician’s viewpoint. *J Clin Oncol* 2021; 39: 238–248.