Is the Ki-67 labelling index ready for clinical use?

Cancer is one of the leading causes of death worldwide. Identification of biomarkers that can detect cancer early, monitor disease progression or serve as a surrogate marker for prognosis and prediction will enable us to personalise medicine and improve care of cancer patients. Up to now, the leading parameters that define treatment recommendations in early breast cancer are estrogen receptor (ER), progesterone receptor, and human epidermal growth factor status. In the last years, global gene expression analysis studies have demonstrated the prime role of proliferation signatures in breast cancer prognosis and prediction of response to therapy [1–3]. This could also be shown in a recent meta-analysis of publicly available BC gene expression studies that revealed that the key biological drivers in nine prognostic signatures were proliferation-related genes, in addition to ER signalling and Her2/neu amplification [4].

Using the information provided by expression data, commercially available multigene assays like the Oncotype DX gene test were developed in which 5 of the 16 genes, used in the text, reflect the proliferative status of the tumour. These specific genes, which include Ki-67, are heavily weighted in the formula used to calculate the test’s recurrence score [5]. Two large randomised studies, one led by the Eastern Cooperative Oncology Group called Tailorx study and the MINDACT (microarray in node negative disease may avoid chemotherapy) trial coordinated by the Breast International Group, are assessing the role of different multigene assays in determining the benefit of chemotherapy in addition to endocrine treatment in node-negative hormone-positive tumours. The panel of the St Gallen International Expert Consensus on the primary therapy of early breast cancer recommends the use of proliferation markers (e.g. Ki-67 index and mitosis) and multigene assays when choosing the appropriate systemic treatment in addition to traditional parameters, such as stage, grade, and endocrine status [6]. A search on the Web site of the American Society of Clinical Oncology revealed that the guidelines for use of biological markers in breast cancer do not include Ki-67 in the list of required routine markers [7].

The Ki-67 antigen was originally identified by a German group [8] in the early 1980s, by use of a mouse mAb against a nuclear antigen from a Hodgkin’s lymphoma-derived cell line. This non-histone protein was named after the researchers’ location, Ki for Kiel University, Germany, with the 67 label referring to the clone number on the 96-well plate [8]. Studies have identified the involvement of Ki-67 in the early steps of polymerase I-dependent ribosomal RNA synthesis. Although it seems that the protein has an important function in cell division, its exact role is still obscure and there is little published work on its overall function [9,10]. Ki-67 expression varies throughout the different cell cycle phases. Cells express the antigen during G1, S, G2, and M phases but not during the resting phase G0. Ki-67 levels are low in the G1 and S phases and rise to their peak level in mitosis. Later in the mitotic phase (anaphase and telophase), a sharp decrease in Ki-67 levels occurs [11]. Since the description of Ki-67, several antibodies, such as MM-1, Ki-S5, and SP6, have been assessed on paraffin sections after antigen retrieval. Mostly, Ki-67 is measured on paraffin sections by an immunohistochemical method, using the MIB-1 antibody. In general, the Ki-67 score is defined as the percentage of total number of tumour cells with nuclear staining.

The use of Ki-67 as a predictive and prognostic marker in breast cancer has been widely investigated. Recent data suggest that a Ki-67 level above 10%–14% defines a high-risk group in terms of prognosis, and according to Yerushalmi et al. [12], acceptance of this definition might make comparisons of future studies more reliable. Therefore, the panel of experts at the St Gallen Consensus in 2009 considered the Ki-67 labelling index important for selecting the addition of chemotherapy to endocrine therapy in hormone receptor-positive breast cancers and classified tumours as low, intermediate, and highly proliferating according to the value of Ki-67 labelling index of ≤15%, 16%–30%, and >30%, respectively.

Neoadjuvant chemotherapy for breast cancer is considered to be the most practical in vivo chemosensitivity test and is ideal for the evaluation of the predictive factors for molecular markers, as tumour tissue can be obtained before and after treatment. Nishimura et al. [13] suggest that the Ki-67 value before neoadjuvant chemotherapy is a strong predictive factor for the effectiveness of the therapy. They found that the pathological documented response was significantly associated with Ki-67 values using multivariate analysis. A higher Ki-67 reveals a higher pathological complete response (pCR) rate. Patients with higher cell proliferation (Ki-67 > 25%) may be better candidates for neoadjuvant chemotherapy. After neoadjuvant chemotherapy, lower Ki-67 values indicate a low chance for pathological complete response but a better prognosis.

DeCensi et al. [14] in this issue of Annals of Oncology analysed the follow-up of a previous study where the effects of different doses of tamoxifen on breast cancer proliferation were studied using Ki-67 expression as the main surrogate end point marker. The change in Ki-67 expression induced by lower doses of tamoxifen was comparable with that achieved with the standard dose, implying that tamoxifen at low doses retains antiproliferative activity [15]. The findings of Dowsett et al. [16] that higher Ki-67 labelling index after 2 weeks of neoadjuvant treatment with either tamoxifen or anastrozole or their combination was associated with shorter recurrence-free survival (RFS) after a median of 37 months in 158 patients.
postmenopausal women with operable or locally advanced breast cancer participating in the IMPACT (Immediate Preoperative Anastrozole Tamoxifen or Combined with Tamoxifen) trial and of Ellis et al. [17] on postmenopausal patients substantiated the correlation of Ki-67 values as a reliable surrogate biomarker of disease outcome. According to the authors, a substantial reduction of Ki-67 labelling index after a short-term challenge test of a few weeks of hormonal treatment might be a simple and inexpensive way to select women with ER-positive breast cancer who may not benefit from adjuvant chemotherapy. Compared with the costs of various multigene assays, testing of Ki-67 would be cost saving and economical at US$30 per test, especially because it can be done in parallel with other immunohistochemical markers and included in the initial pathology report of the core biopsy or surgical specimen [12].

The comparison of baseline Ki-67 labelling index and post-treatment level will enhance the informative value of the test. Baseline Ki-67 values were higher in patients with triple-negative tumours and on the other hand, patients with ER+ and/or PgR+ tumours had lower Ki-67 values. Nishimura et al. [13] found that Ki-67 values before neoadjuvant chemotherapy could predict the effectiveness of the treatment and those after neoadjuvant treatment could predict the disease-free survival (DFS) in patients. The authors of the accompanied paper find the same correlation. They conclude that their findings indicate that Ki-67 labelling index response after short-term presurgical tamoxifen is a better predictor of invasive DFS and overall survival than baseline Ki-67 labelling index in women with ER-positive breast cancer. This finding was also noted by the IMPACT Trialists Group [16], which reported that the continuous evaluation of the changes in Ki-67 values 2 weeks after the initial endocrine treatment improved the prediction for RFS. Therefore, in line with the reports of other groups, the authors stated that the findings support the notion that post-treatment Ki-67 labelling index is a reliable marker of endocrine responsiveness that can allow selection of endocrine-responsive patients who may not benefit from additional chemotherapy.

One of the restrictions to the statement was also mentioned by the authors. They note that given the limited number of events, especially for overall death that was based on 10 events only, their findings should be interpreted with caution. The other limitations in the reported and other studies is the relatively small number of cases analysed leading mostly to higher confidence intervals and the lack of standardisation of Ki-67 pathological assessment. We are in line with Colloza et al. [18] that despite the absence of standardisation of Ki-67 pathological assessment, the acceptance of Ki-67 as a standard marker requires much more research to be done. Further analysis using validated methods is necessary before its widespread adoption and only a complete standardisation of tissue handling and processing will improve the value of Ki-67 as a clinically useful and widely accepted marker [18]. Nevertheless, the MIB1 labelling index assay is accepted to be a low cost simple method, which is perfectly suitable for standardisation in clinical laboratory practice.

Another possible marker to monitor proliferation in a tumour tissue would be the Ki-S2 antibody. This antibody recognises a 100-kd proliferation-specific nuclear protein expressed exclusively in the cell cycle phases S, G2, and M. Therefore, actively proliferating cells that constitute a subset of the population recognised by Ki-67 were specifically labelled. The cycling ratio, defined as the ratio of the Ki-S2 (p100) labelling index to the Ki-67 labelling index, represents the relative fraction of cells in the S, G2, and M phases of the cell cycle. This finding implies that alterations of cell cycle regulation at the G1–S transition strongly influence breast cancer progression [19]. Prognosis is apparently best indicated by the percentage of cells in S through M phases of the cell cycle. Measurement of the Ki-S2 labelling index of a tumour sample also may improve a clinician’s ability to make an accurate prognosis and to identify patients with a low risk of recurrence who may not need adjuvant therapy [20].

Concerning Ki-67 antibodies, recently a comparative study has been published revealing that the antibody SP6 might be better suited for image analysis [21].

In summary, cellular proliferation is a major determinant of the biologic behaviour of breast cancer and a standardisation of the techniques to determine cellular proliferation is needed.

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disclosure

The authors declare no conflict of interest.

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