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Predictive Value of Tumor Ki-67 Expression in Two Randomized Trials of Adjuvant Chemoendocrine Therapy for Node-Negative Breast Cancer

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On the behalf of the International Breast Cancer Study Group

Several small studies have reported that having a high percentage of breast tumor cells that express the proliferation antigen Ki-67 (ie, a high Ki-67 labeling index) predicts better response to neoadjuvant chemotherapy. However, the predictive value of a high Ki-67 labeling index for response to adjuvant chemotherapy is unclear. To investigate whether Ki-67 labeling index predicts response to adjuvant chemoendocrine therapy, we assessed Ki-67 expression in tumor tissue from 1924 (70%) of 2732 patients who were enrolled in two randomized International Breast Cancer Study Group trials of adjuvant chemoendocrine therapy vs endocrine therapy alone for node-negative breast cancer. A high Ki-67 labeling index was associated with other factors that predict poor prognosis. Among the 1521 patients with endocrine-responsive tumors, a high Ki-67 labeling index was associated with worse disease-free survival but the Ki-67 labeling index did not predict the relative efficacy of chemoendocrine therapy compared with endocrine therapy alone. Thus, Ki-67 labeling index was an independent prognostic factor but was not predictive of better response to adjuvant chemotherapy in these studies.

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The percentage of proliferating cells in a tumor (ie, the tumor proliferation fraction) is an established predictor of breast cancer prognosis (1,2). The proliferation antigen Ki-67 is detectable in cells at all phases of the cell cycle except G_0 (3), and the Ki-67 labeling index (the percentage of cells with Ki-67–positive nuclear immunostaining) is a measure of tumor proliferation (4,5) that has been associated with breast cancer outcome in several studies (6–10). Other studies (11,12) have suggested that a high Ki-67 labeling index is predictive of responsiveness to neoadjuvant (primary) chemotherapy, but, to our knowledge, there are no such reports concerning Ki-67 labeling index as a predictor of responsiveness to adjuvant chemotherapy.

In 2005, the ninth St Gallen consensus conference on primary therapy for early breast cancer (13) emphasized the importance of the endocrine responsiveness of the tumor in selecting adjuvant therapy for early breast cancer and acknowledged the existence of a group of patients whose responsiveness to endocrine therapy is uncertain even though their tumors express hormone receptors. Because these patients may benefit from chemoendocrine therapy, we examined whether the Ki-67 labeling index could identify patients who might particularly benefit from the addition of chemotherapy to endocrine therapy in the adjuvant setting in two International Breast Cancer Study Group (IBCSG) trials.

IBCSG Trials VIII (14) and IX (15) were randomized clinical trials that were conducted between 1988 and 1999; the median follow-up for each trial is 10 years. The trials compared adjuvant endocrine therapy alone with sequential chemotherapy followed by endocrine therapy for lymph node–negative invasive breast cancer in premenopausal (Trial VIII) and postmenopausal (Trial IX) women. Trial VIII (14) evaluated whether sequential treatment with six 28-day courses of combination chemotherapy with cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) followed by 18 monthly subcutaneous implants of goserelin (CMF→goserelin) improved disease-free survival compared with either six 28-day courses of CMF alone or 24 monthly implants of goserelin alone. Trial IX (15) evaluated whether sequential treatment with three 28-day courses of CMF followed by tamoxifen for 57 months (CMF→tamoxifen) improved disease-free survival compared with tamoxifen alone for 60 months. During

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CONTEXT AND CAVEATS

Prior knowledge

Some studies have suggested that having a high percentage of breast tumor cells that label with an antibody against the proliferation antigen Ki-67 predicts a better response to primary (ie, neoadjuvant) chemotherapy.

Study design

A retrospective assessment of the predictive value of a high Ki-67 labeling index for response to therapy among women enrolled in two randomized trials of adjuvant chemoendocrine therapy vs endocrine therapy alone for node-negative breast cancer.

Contribution

A high Ki-67 labeling index did not predict which women would benefit from further treatment with chemotherapy added to endocrine therapy.

Limitations

Only women with node-negative breast cancer were included in this study.

Implications

Other biomarkers are needed to define which women with endocrine-responsive node-negative early breast cancer could benefit from the addition of adjuvant chemotherapy to endocrine therapy.

the conduct of these trials, the estrogen receptor (ER) and the progesterone receptor (PgR) status of the tumor and tumor size and grade were locally assessed and noted on trial case report forms. Tumors were graded 1–3 either according to Bloom and Richardson (16) or according to overall differentiation as well differentiated (grade 1), moderately differentiated (grade 2), or poorly differentiated (grade 3).

In 2003, the IBCSG completed the retrospective collection of formalin-fixed, paraffin-embedded primary breast tumor tissue samples that were obtained from Trials VIII and IX participants. The collection program was conducted in accordance with institutional guidelines and national laws. The samples were subjected to immunohistochemical assessment of ER, PgR, and HER2 expression and Ki-67 labeling index at the IBCSG Central Laboratory in Milan, Italy, by personnel who were blinded to participant treatment assignment and outcomes, as previously described (17). Whole tumor sections were incubated with the specific primary mouse monoclonal antibodies to ER (clone 1D5, 1:100 dilution) or PgR (clone 1A6, 1:800 dilution) (both from Dako, Glostrup, Denmark). HER2 immunoreactivity was assessed using a HercepTest kit (Dako) as recommended by the manufacturer and scored for the intensity of immunostaining, the completeness of cell membrane staining, and the percentage of immunoreactive neoplastic cells by using a four-tier scale from 0 to 3+, as previously described (18). The centrally assessed values of ER, PgR, and HER2 status were used in this report.

Reassessment of the trial conclusion based on the centrally assessed hormone receptor values confirmed the finding that the benefit of chemotherapy was limited to patients whose breast tumors expressed little or no ER or PgR (17,19).

Tumor material was available and assessable for Ki-67 labeling index for 758 (71%) of 1063 Trial VIII patients and 1166 (70%) of 1669 Trial IX patients. Ki-67 labeling index was assessed using mouse monoclonal antibody MIB-1 (1:200 dilution; Dako); the percentage of cells that showed definite nuclear immunoreactivity with MIB-1 among 2000 invasive neoplastic cells in randomly selected high-power (×400) fields at the periphery of the tumor was recorded.

Centrally reviewed ER and PgR status were classified as present (≥1% immunoreactive cells) or absent (<1% immunoreactive cells). Samples were considered to be positive for HER2 overexpression if the staining intensity score was 3+ and negative for HER2 overexpression if the staining intensity score was 0, 1+, or 2+ (20). The Ki-67 labeling index was dichotomized to high (≥19% immunoreactive cells) and low (<19% immunoreactive cells) groups by using the median value of Ki-67 immunoreactivity as the cut point, which was based on the frequency distributions of the Ki-67 labeling index in the two trial cohorts (Fig. 1, A and B).

Univariate and multivariable logistic regression modeling was used to assess the association of other tumor features with high vs low Ki-67 labeling index. Analyses were undertaken separately for the two trials. These analyses revealed that in both trials, higher tumor grade, larger tumor size, and the absence of tumor expression of ER and of PgR were associated with a high Ki-67 labeling index ($P < .001$ for each) (Supplementary Table 1, available online). HER2 overexpression was associated with a high Ki-67 labeling index in postmenopausal patients (ie, Trial IX; $P < .001$) but not in premenopausal patients (ie, Trial VIII; $P = .61$) (Supplementary Table 1, available online).

We next examined the association of high and low Ki-67 labeling indices with disease-free survival among patients with endocrine-responsive breast cancer (ie, 923 patients with ER-present tumors on Trial IX and 598 patients with ER- and/or PgR-present tumors on Trial VIII). Cox proportional hazards modeling was used to examine interactions of Ki-67 labeling index and other tumor characteristics with disease-free survival. To check assumptions of proportionality, curves of the log of the cumulative hazard function for each value of a covariate adjusted for other covariates in the model were plotted and assessed visually to determine if the vertical shift between the curves was constant over time. The data appeared to meet the assumptions of proportionality. All P values are two-sided, and statistical significance was defined as P less than or equal to .05.

Among postmenopausal patients who were treated in Trial IX, a high Ki-67 labeling index was associated with worse disease-free survival (hazard ratio [HR] of recurrence or death = 1.60, 95% confidence interval [CI] = 1.26 to 2.03, $P < .001$). A multivariable analysis adjusting for other tumor features confirmed that high Ki-67 labeling index was an independent prognostic feature ($P \leq .05$) (data not shown). We examined the pairwise interactions of the other tumor features and Ki-67 labeling index with disease-free survival to investigate whether the association between Ki-67 labeling index and disease-free survival varied as a function of specific tumor characteristics. There was no evidence of an interaction for any factor: a high Ki-67 labeling index was consistently associated with worse disease-free survival (Fig. 2). As was previously reported for Trial IX as a whole (15), treatment arm was not associated with disease-free survival in this endocrine-responsive subgroup of patients. There was no interaction of Ki-67 labeling index and treatment arm with disease-free survival ($P_{interaction} = .45$; Fig. 2), indicating that patients whose tumors had a high Ki-67 labeling index had worse disease-free

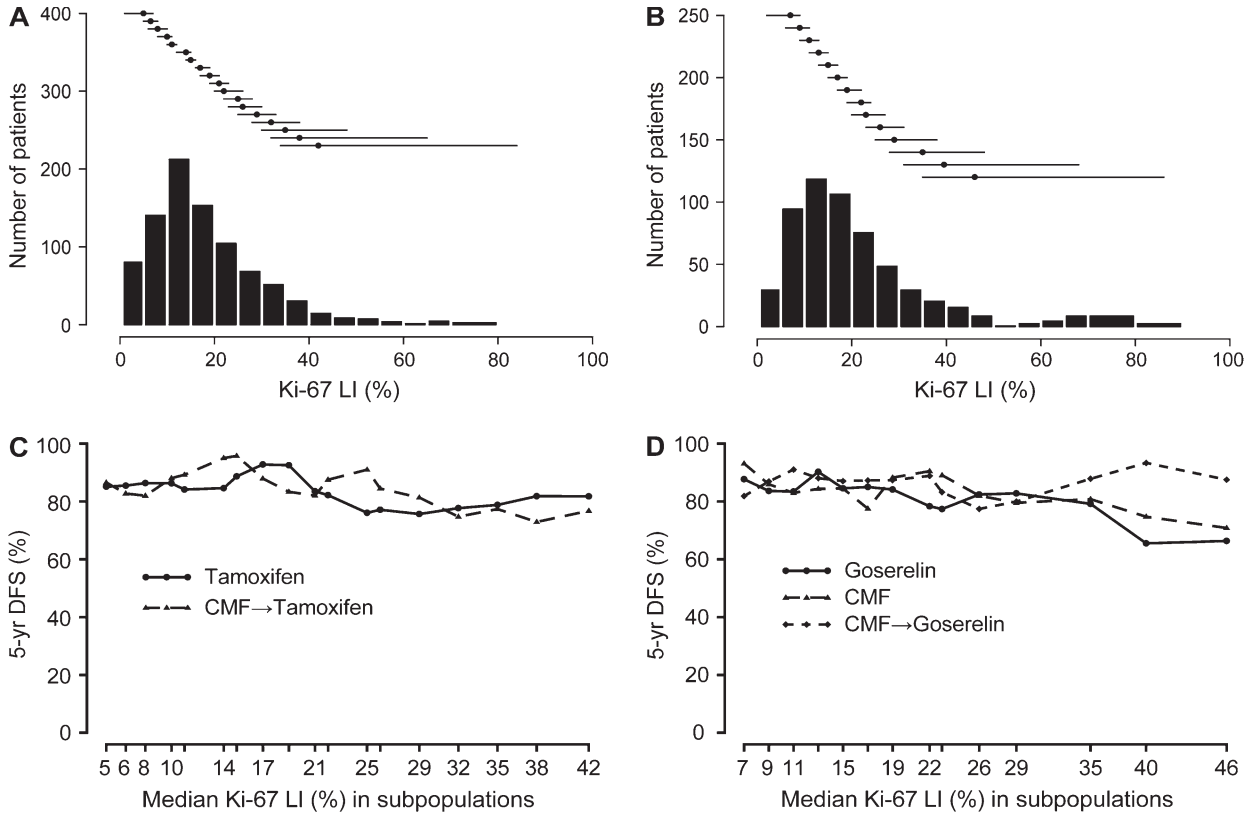

Fig. 1. Distribution and subpopulation treatment effect pattern plots (STEPP) analysis of breast cancer tumor Ki-67 labeling index. Frequency distribution of Ki-67 labeling index in postmenopausal patients (Trial IX) with estrogen receptor (ER)–expressing tumors (**A**) and in premenopausal patients (Trial VIII) with ER- and/or progesterone receptor (PgR)–expressing tumors (**B**). The **black circles** indicate the median Ki-67 labeling index, and the **horizontal lines** indicate the range for subpopulations of patients that were used for the STEPP analysis. **C** and **D**) STEPP analysis of 5-year disease-free survival by treatment arm according to Ki-67 labeling index in postmenopausal patients (Trial IX) with ER-expressing tumors (**C**) and in premenopausal patients (Trial VIII) with ER- and/or PgR-expressing tumors (**D**). Overlapping subpopulations of patients were defined on the basis of Ki-67 labeling index, and the resulting patterns of the treatment effects estimated within each subpopulation are displayed. The subpopulations have a fixed number of patients (approximately 120 for Trial IX and approximately 100 for Trial VIII); each subsequent subpopulation changed by 20 patients. The x-axis indicates the median Ki-67 labeling index value for patients in each subpopulation; the y-axis indicates the treatment effects, expressed as the 5-year disease-free survival percentage estimated by using the Kaplan-Meier method. DFS = disease-free survival; LI = labeling index; CMF = combination chemotherapy with cyclophosphamide, methotrexate, and 5-fluorouracil.

survival than patients whose tumors had a low Ki-67 labeling index regardless of the treatment they received. The relative treatment effect of CMF→tamoxifen vs tamoxifen alone among patients whose tumors had a high Ki-67 labeling index (HR = 1.03, 95% CI = 0.73 to 1.45) was consistent with that among patients whose tumors had a low Ki-67 labeling index (HR = 0.86, 95% CI = 0.61 to 1.20). This homogeneity in the treatment effect was confirmed in a multivariable analysis that adjusted for tumor grade, tumor size, and PgR and HER2 status: in patients with ER-expressing tumors, a high Ki-67 labeling index did not predict resistance to tamoxifen alone; nor did it predict benefit from sequential CMF→tamoxifen ($P_{interaction}$ = .41).

Among premenopausal patients who were treated in Trial VIII, high Ki-67 labeling index was associated with worse disease-free survival (HR of recurrence or death = 1.66, 95% CI = 1.20 to 2.29; P = .002). A multivariable analysis that adjusted for other tumor features confirmed that a high Ki-67 labeling index was an independent prognostic marker (P < .05) (data not shown). We examined the pairwise interactions of other tumor features and Ki-67 labeling index with disease-free survival. There was no evidence of an interaction for any factor: a high Ki-67 labeling index was consistently associated with worse disease-free survival (Fig. 2). As was previously reported for Trial VIII as a whole (14), treatment arm was not associated with outcome in this endocrine-responsive subgroup of patients. There was no interaction of Ki-67 labeling index and treatment arm with disease-free survival ($P_{interaction}$ = .90; Fig 2), indicating that patients whose tumors had a high Ki-67 labeling index had worse disease-free survival than patients whose tumors had low Ki-67 labeling index regardless of treatment received. The relative treatment effect of each pairwise comparison of the three treatment arms among patients whose tumors had a high Ki-67 labeling index was consistent with that among patients with low tumor Ki-67 labeling index (CMF→goserelin vs goserelin alone in patients with low [HR = 0.78, 95% CI = 0.44 to 1.40] and high [HR = 0.81, 95% CI = 0.48 to 1.38] Ki-67 labeling index; CMF→goserelin vs CMF in patients with low [HR = 1.05, 95% CI = 0.56 to 1.99] and high [HR = 0.92, 95% CI = 0.55 to 1.52] Ki-67 labeling index; CMF vs goserelin in patients with low [HR = 0.74, 95% CI = 0.41 to 1.35] and high

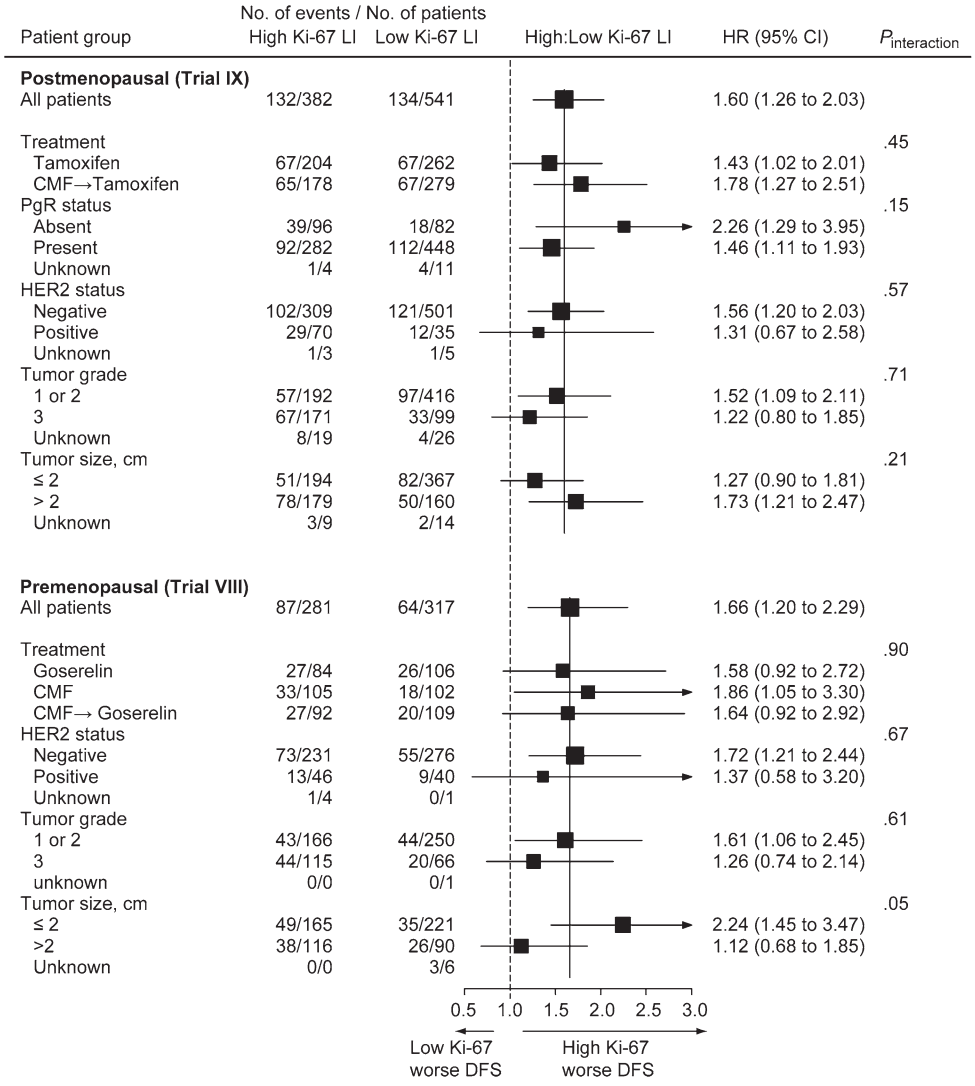

Fig. 2. Association between Ki-67 labeling index and disease-free survival according to other tumor features among postmenopausal patients (Trial IX) and premenopausal patients (Trial VIII) with endocrine-responsive tumors. The **box** size is inversely proportional to the SE of the hazard ratio (HR); the extending **horizontal lines** indicate the 95% confidence intervals (CIs); an arrow indicates that the confidence interval extends beyond the limits of the x-axis. The **vertical solid lines** provide a reference for the hazard ratio of the overall cohorts, and the **dashed line** provides a reference for hazard ratio = 1.0. Disease-free survival was defined as the length of time from the date of randomization to any relapse (including ipsilateral breast recurrence), the appearance of a second primary cancer (including contralateral breast cancer), or death, whichever occurred first. Cox proportional hazards modeling was used to estimate hazard ratios and 95% confidence intervals and two-sided *P* values for pairwise interactions. Unknown values are excluded from *P* value calculations. LI = labeling index; CMF = cyclophosphamide, methotrexate, fluorouracil chemotherapy; PgR = progesterone receptor; DFS = disease-free survival.

[HR = 0.89, 95% CI = 0.53 to 1.48] Ki-67 labeling index). This homogeneity in the treatment effects was confirmed in a multivariable analysis that adjusted for tumor grade, tumor size, and PgR and HER2 status: in patients with ER-expressing tumors, a high Ki-67 labeling index did not predict resistance or benefit to any of the treatments ($P_{interaction}$ = .69).

We further examined the pattern of treatment effects across the continuum of Ki-67 labeling indices using the nonparametric subpopulation treatment effect pattern plot (STEPP) method (21), which avoids the need to select a cut point in the distribution of a continuous feature such as Ki-67 labeling index. The STEPP method uses a sliding-window approach to define several overlapping subpopulations of patients according to Ki-67 labeling index and plots the resulting treatment effects estimated within each subpopulation. The subpopulations have a fixed number of patients (approximately 120 for Trial IX and approximately 100 for Trial VIII); each subsequent subpopulation changed by 20 patients. The plot's x-axis indicates the median Ki-67 labeling index for patients in each subpopulation; the y-axis indicates the treatment effects, expressed as the 5-year disease-free survival percentage estimated by using the Kaplan-Meier method. We observed no evidence of any association between Ki-67 labeling index and the relative efficacy of the trial therapies across the continuum of Ki-67 labeling indices (Fig. 1, C and D).

Our primary goal was to determine whether the Ki-67 labeling index of a tumor can be used to identify endocrine-responsive breast cancer patients who would benefit from adjuvant chemotherapy. Ki-67 has been previously evaluated as a prognostic factor (6–10), and our finding that Ki-67 is a prognostic factor in early breast cancer is, in general, consistent with the conclusions of a recent meta-analysis (22) that included more than 12 000 patients and our previous study (9) and those of others (2,6–8,10,23) that suggest that higher values of Ki-67 indicate a worse prognosis.

Several other studies have examined the value of using tumor Ki-67 expression to predict response to neoadjuvant chemotherapy. Chang et al. (11) and Archer et al. (12) reported an association between high pretreatment Ki-67 labeling index and better response to chemotherapy in the neoadjuvant setting. A 2005 review article (24) cited five small studies (the number of patients per study ranged from 106 to 399) (25–29) that investigated the predictive value of Ki-67 labeling index in

the neoadjuvant setting; two of these studies (25,26) concluded that a high Ki-67 labeling index is associated with response to chemotherapy, whereas the other three studies (27–29) found no such association.

Our data indicate that Ki-67 labeling index does not predict which patients will benefit from adding chemotherapy to endocrine therapy in the adjuvant setting. Instead, our data indicate that a high Ki-67 labeling index is associated with worse disease-free survival in all treatment groups and that the association between type of treatment and disease-free survival is independent of Ki-67 labeling index. Thus, in this study, Ki-67 labeling index was a prognostic factor, not a predictive factor.

A limitation of this study is that it included only patients with node-negative breast cancer; results may differ in other populations.

Our results suggest that although tumor proliferation fraction as assessed by Ki-67 labeling index is a valuable prognostic indicator, other biomarkers will be required to define which patients with endocrine-responsive, node-negative early breast cancer would benefit from the addition of adjuvant chemotherapy to endocrine therapy.

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The authors and the IBCSG were responsible for the trial design and conduct; collection, analysis, and interpretation of data; writing of the manuscript; and the decision to submit the manuscript for publication.

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