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ORIGINAL REPORT

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

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The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

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ABSTRACT

Purpose
To evaluate the prognostic and predictive value of Ki-67 labeling index (LI) in a trial comparing letrozole (Let) with tamoxifen (Tam) as adjuvant therapy in postmenopausal women with early breast cancer.

Patients and Methods
Breast International Group (BIG) trial 1-98 randomly assigned 8,010 patients to four treatment arms comparing Let and Tam with sequences of each agent. Of 4,922 patients randomly assigned to receive 5 years of monotherapy with either agent, 2,685 had primary tumor material available for central pathology assessment of Ki-67 LI by immunohistochemistry and had tumors confirmed to express estrogen receptors after central review. The prognostic and predictive value of centrally measured Ki-67 LI on disease-free survival (DFS) were assessed among these patients using proportional hazards modeling, with Ki-67 LI values dichotomized at the median value of 11%.

Results
Higher values of Ki-67 LI were associated with adverse prognostic factors and with worse DFS (hazard ratio [HR; high:low] = 1.8; 95% CI, 1.4 to 2.3). The magnitude of the treatment benefit for Let versus Tam was greater among patients with high tumor Ki-67 LI (HR [Let:Tam] = 0.53; 95% CI, 0.39 to 0.72) than among patients with low tumor Ki-67 LI (HR [Let:Tam] = 0.81; 95% CI, 0.57 to 1.15; interaction $P = .09$).

Conclusion
Ki-67 LI is confirmed as a prognostic factor in this study. High Ki-67 LI levels may identify a patient group that particularly benefits from initial Let adjuvant therapy.

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INTRODUCTION

Recent St Gallen Guidelines for the selection of therapy in early breast cancer have increasingly stressed the importance of identifying primarily factors predictive of response to particular therapies, and secondarily prognostic factors for risk of recurrence.[1] Tumor proliferation fraction is an established predictor of prognosis.[2] The nuclear protein Ki-67, present in cycling cells,[3] is an indicator of tumor proliferation[4,5] and has been found to be a prognostic marker in breast cancer.[6-11] High Ki-67 labeling index (LI) is reportedly predictive of responsiveness to preoperative chemotherapy.[12,13] A fall in Ki-67 LI during preoperative endocrine therapy has been associated with pathologic tumor response,[14] whereas Dowsett et al[15] found that persistently higher Ki-67 LI after short-term preoperative endocrine therapy predicted shorter disease-free survival. We have recently described the role of Ki-67 LI as a prognostic factor in premenopausal and postmenopausal women with hormone receptor–positive, node-negative breast cancer, but we did not find Ki-67 LI to be predictive of differential responsiveness to the chemoendocrine or endocrine therapies studied in that adjuvant setting.[16] To our knowledge, there are no reports of Ki-67 LI predicting responsiveness to postoperative cytotoxic or endocrine adjuvant therapies.

Breast International Group (BIG) trial 1-98 is an international, double-blind, four-arm, randomized phase III trial investigating the aromatase inhibitor letrozole (Let) compared with tamoxifen (Tam) in the adjuvant setting among postmenopausal women with endocrine-responsive, early invasive breast cancer. Both the primary analysis[17] and a subsequent report limited to patients randomly assigned to the monotherapy treatment arms[18] supported the improvement of disease-free survival (DFS) in patients assigned initial Let compared with Tam.

The purpose of this report is to examine the value of Ki-67 LI, as assessed in the International Breast Cancer Study Group (IBCSG) Central Pathology Laboratory, both as a prognostic factor and as a predictive factor for differential efficacy of Let versus Tam used as initial adjuvant therapy in postmenopausal women with endocrine-responsive breast cancer.

PATIENTS AND METHODS

Study Design

The BIG 1-98 patient population was defined as postmenopausal women with early invasive breast cancer whose tumors were assessed by local pathologists as hormone receptor (estrogen receptor [ER] and/or progesterone receptor [PgR]) positive. Between March 1998 and March 2000, patients were randomly assigned to receive adjuvant endocrine therapy in one of the monotherapy arms comprising either Let 2.5 mg/d or Tam 20 mg/d for 5 years, and from April 1999 to May 2003 to all four arms including the sequence of 2 years, Tam followed by 3 years of Let or 2 years of Let followed by 3 years of Tam. The primary efficacy analysis among 8,010 patients[17] was updated as specified by protocol, and reported among the 4,922 patients who were randomly assigned to the monotherapy arms only at a median follow-up time of 51 months.[18] This updated analysis, limited to patients assigned to 5 years of monotherapy with either Tam or Let, is used for the current report. Retrospective tissue collection was carried out in accordance with institutional guidelines and national laws. Tumor material from 2,906 (59%) of the 4,922 patients was submitted to the IBCSG Central Pathology Office for central pathology review (CPR) of Ki-67. The analysis cohort was further limited to patients for whom adequate tumor material was available, and for whom CPR confirmed expression of ER in the tumor (n = 2,685; Fig 1).

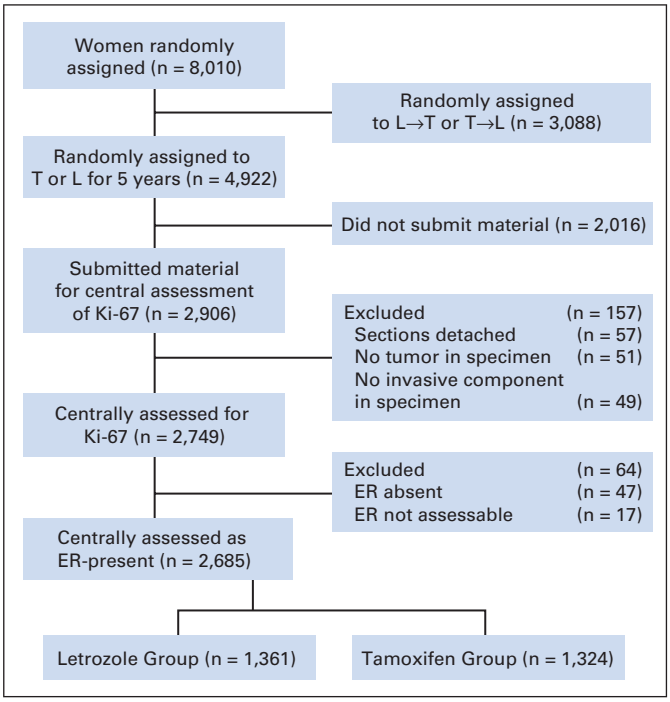

Fig 1. Patients from the Breast International Group 1-98 trial included and excluded in this study according to treatment group and availability of tumor material. L, letrozole; T, tamoxifen; ER, estrogen receptor.

Pathology

The IBCSG Central Pathology Laboratory performed central review of paraffin-embedded primary tumor specimens for ER and PgR by immunohistochemistry (IHC),[19] and for human epidermal growth factor receptor 2 (HER-2) by IHC and fluorescent in situ hybridization (FISH).[20] Tumors were considered to express ER or PgR if they showed at least 1% of immunoreactive cells. Tumors were considered to be HER-2 positive if amplified by FISH, or in a few cases with nonassessable FISH results, if IHC was 3+.

Ki-67 was assessed by IHC using the Mib-1 monoclonal antibody (1:200 dilution; Dako, Glostrup, Denmark). Slides were cut and stained centrally using an automated immunostainer (Autostainer, Dako), and the results assessed without the use of an image analysis system. The percentage of cells showing definite nuclear immunoreactivity among 2,000 invasive neoplastic cells in randomly selected, high-power (magnification, ×400) fields at the periphery of the tumor was recorded. The CPR was performed without knowledge of patients' treatment assignment or outcome. All of the assays were performed on whole tissue sections.

End Points and Statistical Considerations

A comparison of patients with and without material for CPR was previously described.[21] Levels of Ki-67 LI were dichotomized as high (> 11%) and low (≤ 11%) for the primary analysis. The cutoff is the median of the distribution of Ki-67 LI among all BIG 1-98 trial patients' material that was centrally assessed for Ki-67 (n = 4,399 of 8,010). Associations of other prognostic tumor features and Ki-67 LI levels were evaluated using χ^2 tests.

The protocol-specified primary trial end point was DFS, which was defined as the time from random assignment to the earliest time of invasive recurrence in local, regional, or distant sites; a new invasive breast cancer in the contralateral breast; any second (nonbreast) malignancy; or death resulting from any cause. The distribution of DFS was summarized using the Kaplan-Meier method.[22] Proportional hazards modeling,[23] stratified by whether chemotherapy had been administered and by randomization option (two or four arm),[17] was used to investigate predictors of DFS and to estimate hazard ratios (HRs), 95% CI, and to assess interactions of the treatment effect with Ki-67 LI and other prognostic variables. Subpopulation Treatment Effect Pattern Plot (STEPP)[24] methodology was employed to further illustrate the relationship between Ki-67 LI and outcome across the continuum of Ki-67 LI levels. The STEPP method uses a sliding-window approach to define several overlapping subpopulations of patients according to Ki-67 LI. The values on the *x*-axis are the median values of Ki-67 LI for patients in a subpopulation, and the *y*-axis indicates the treatment effects, expressed as the Kaplan-Meier estimates of 4-year DFS. Each subpopulation contains approximately 200 patients and slides by approximately 50 patients.

To determine the most predictive Ki-67 LI cut point, separate proportional hazards models including treatment, Ki-67 LI, and their interaction as predictors were constructed with Ki-67 LI dichotomized at successive integer values between 1% and 55%. The best cut point was identified by determining the Ki-67 LI division that minimized the Wald χ^2 *P* value of the interaction.

Statistical analyses used SAS version 9.1 (SAS Institute, Cary, NC) and *S*-PLUS version 6.1 (Insightful Corp, Seattle, WA). All statistical tests provided two-sided *P* values, and $P \leq .05$ was considered statistically significant.

RESULTS

In univariate analyses, high (> 11%) Ki-67 LI was associated with larger tumors, higher tumor grade, peritumoral vascular invasion, and HER-2 positivity (each $P < .01$), but in this population, as distinct

from our earlier trials,[16] there was no association between Ki-67 LI and presence or absence of PgR expression (Table 1).

DFS was significantly lower for patients with tumors with high Ki-67 LI (HR [high:low] = 1.8; 95% CI, 1.4 to 2.3; $P = .0001$), confirming the prognostic value of Ki-67 LI in this cohort (Fig 2A). Four-year DFS estimates were 92.2% for low versus 85.6% for high Ki-67 LI. In a multivariable proportional hazards regression model adjusted for patient age, PgR status, tumor size, tumor grade, nodal status, HER-2 status, and presence of peritumoral vascular involvement, high Ki-67 LI remained an independent adverse prognostic factor (HR = 1.4; 95% CI, 1.1 to 1.9; $P = .02$).

In this analytic cohort, as for the trial population as a whole, DFS was significantly better in patients randomly assigned to receive Let compared with Tam; (HR [Let:Tam] = 0.63, 95% CI, 0.50 to 0.80; $P < .0001$) and 4-year DFS estimates were 91.7% and 86.5%, respectively. There was a suggestion of heterogeneity in the treatment effect among patients with tumors having high versus low Ki-67 LI ($P = .09$ for interaction; Fig 2B). Within the subgroup having high tumor Ki-67 LI, the hazard for a DFS event for patients who received Let was approximately half the hazard of patients who received Tam (HR [Let:Tam] = 0.53, 95% CI, 0.39 to 0.72) which was a greater treatment effect than that observed among patients with low tumor Ki-67 LI (HR [Let:Tam] = 0.81; 95% CI, 0.57 to 1.15). The estimated 4-year DFS among patients in the subgroup having high tumor Ki-67 LI who received Let (89.6%) was comparable with those for patients with low Ki-67 LI who received either Let (93.4%) or Tam (90.9%). The overall pattern was similar whether patients had node-negative or node-positive disease or whether the tumors were ER expressing (1% to 79%) or strongly ER expressing (80% or higher; Fig 3).

Table 1. Association of Tumor Ki-67 LI With Other Tumor Features

Feature	Ki-67 LI: Low ≤ 11% No.	%	High > 11% No.	%	*P*
No. of patients	1,433	53.4	1,252	46.6	
HER-2 overexpression					< .0001
No	1396	97.4	1114	89.0	
Yes	37	2.6	138	11.0	
Nodal status					.0004
N-/Nx	971	67.8	767	61.3	
N+	462	32.2	485	38.7	
Tumor size, cm					< .0001
≤ 2	1013	70.7	750	59.9	
> 2	414	28.9	491	39.2	
Unknown	6	.4	11	0.9	
Tumor grade					< .0001
1	561	39.1	192	15.3	
2	655	45.7	632	50.5	
3	89	6.2	326	26.0	
Unknown	128	8.9	102	8.1	
Peritumoral vascular invasion					< .0001
No	1127	78.6	870	69.5	
Yes	178	12.4	278	22.2	
Unknown/not able to assess	128	8.9	104	8.3	
ER/PgR expressed (assessed centrally)*					.68
ER present/PgR absent	149	10.4	119	9.5	
ER present/PgR present	1278	89.2	1129	90.2	
Other	6	.4	4	0.3	

Abbreviations: LI, labeling index; HER-2, human epidermal growth factor receptor 2; ER, estrogen receptor; PgR, progesterone receptor.
*ER and PgR each are considered as present if ≥1% immunoreactive cells.

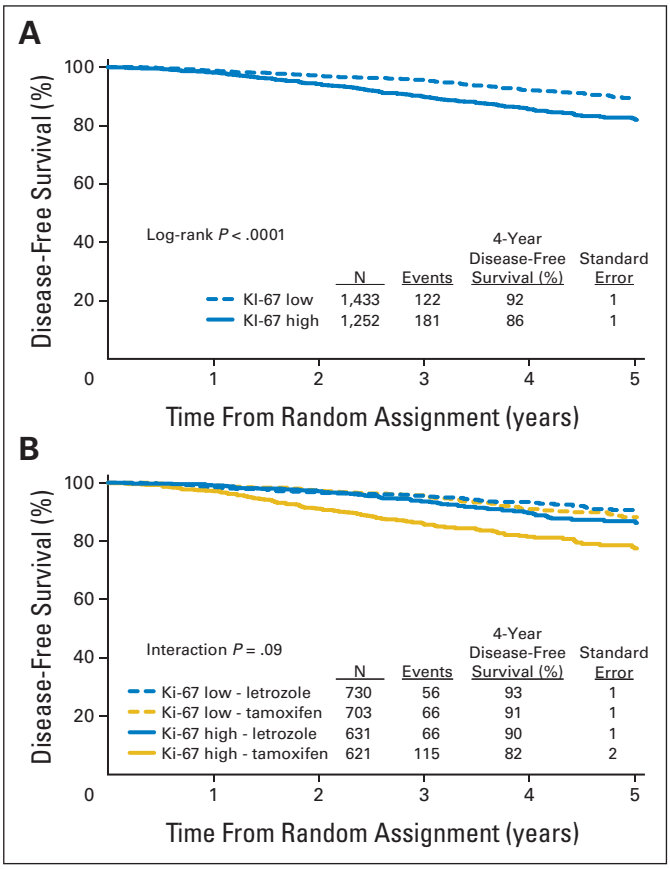

Fig 2. Kaplan-Meier estimates of disease-free survival according to (A) level of Ki-67 labeling index (high > 11% *v* low ≤ 11%) and (B) treatment assignment.

Through exploratory analyses of integer cut points of the Ki-67 LI distribution, the *P* value for the interaction of treatment and Ki-67 LI was minimized ($P = .02$) when Ki-67 LI was dichotomized at 14%. When Ki-67 was dichotomized as less than or equal to 14% versus greater than 14%, treatment comparisons yielded results similar to those reported above.

The STEPP analysis of 4-year DFS across the continuum of Ki-67 LI percentages (Fig 4) displays the suggested heterogeneity in the treatment effect across various levels of Ki-67 LI. For subpopulations with higher median Ki-67 LI greater than 10% (and especially above 30%), the separation of the curves suggests greatest benefit of Let relative to Tam for subpopulations with the highest levels of Ki-67 LI.

As a hypothesis-generating exercise, we further explored whether the suggested difference in the relative efficacy of Let versus Tam for high and low tumor Ki-67 LI might be modified by other prognostic tumor features (Fig 5). There was little evidence for any such interaction, and the overall impression from inspection of the forest plot is that the relative efficacy of Let is greater in subgroups with high tumor Ki-67 LI regardless of other tumor features.

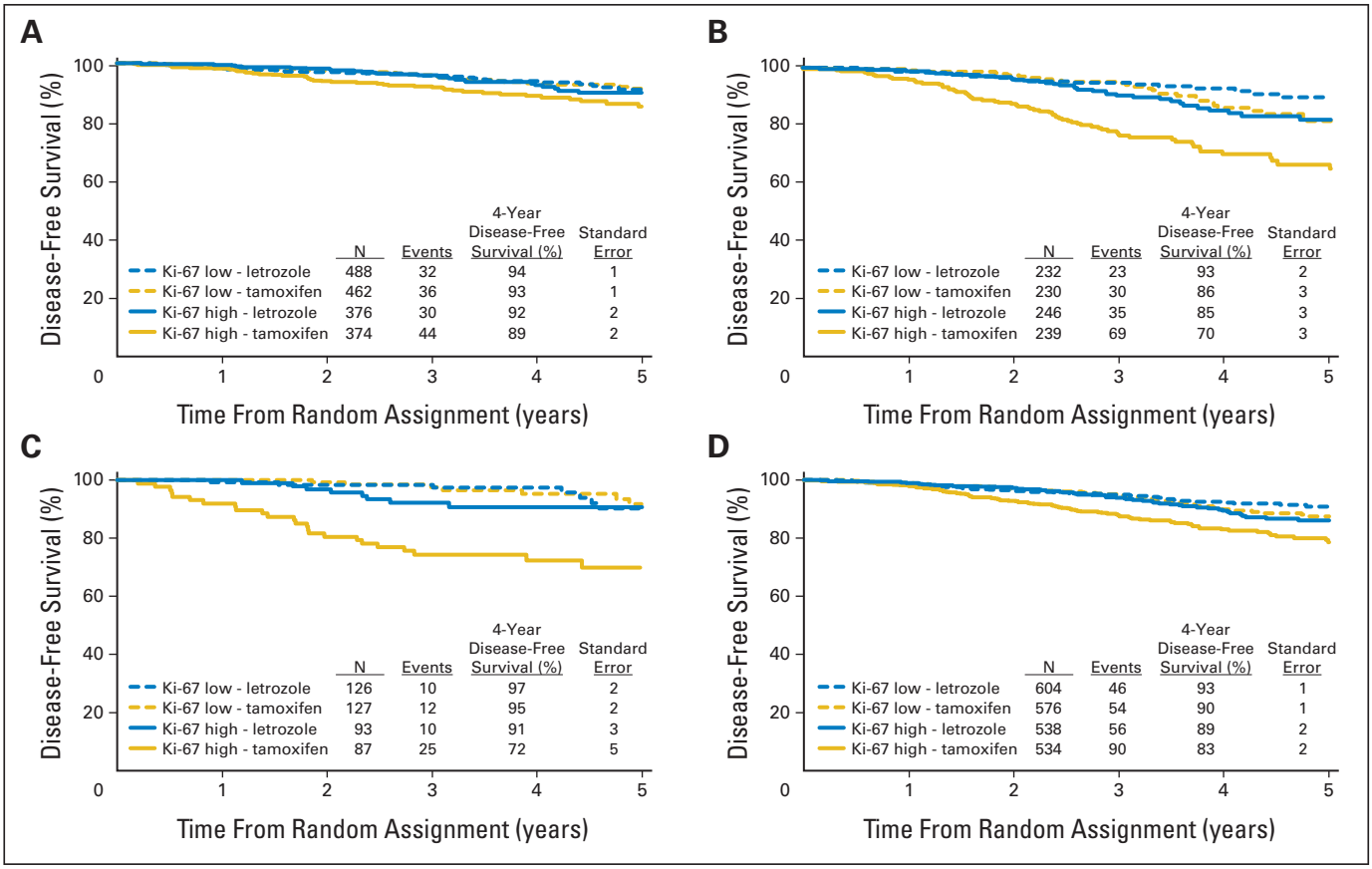

Fig 3. Kaplan-Meier estimates of disease-free survival according to level of Ki-67 labeling index (LI) (high > 11% *v* low ≤ 11%) and treatment assignment, separately for patients (A) with lymph node–negative disease and (B) node-positive disease, and with tumors that are (C) estrogen receptor expressing (1% to 79%) and (D) strongly estrogen receptor expressing (≥ 80%).

DISCUSSION

This analysis supports previous reports that Ki-67 LI is a prognostic factor in early breast cancer.[6-11] The median value of 11% for KI-67 LI in the CPR cohort from BIG 1-98 was lower than the 19% median we had observed in an earlier series in IBCSG Trial IX, which compared chemoendocrine with endocrine therapy among node-negative patients,[16] but similar to the 10% cut point used in several series published by others.[7,25] We[16] and others[6] have noted a correlation between Ki-67 LI and adverse prognostic factors including tumor differentiation, which may partly explain the lower median Ki-67 LI in the present series, because the earlier trials included more patients with high-grade tumors.[16]

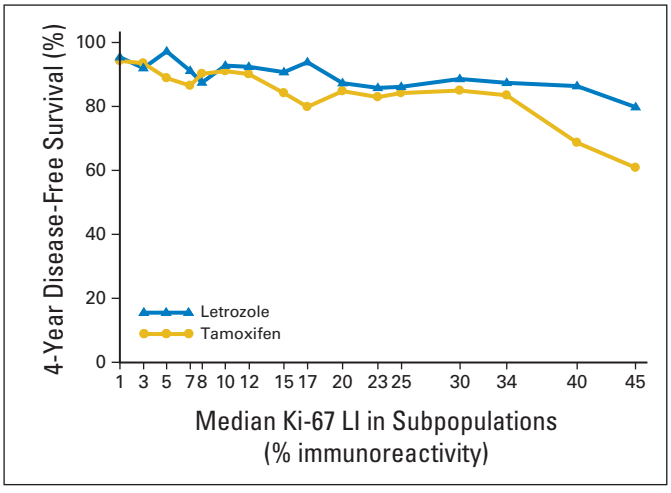

Fig 4. Subpopulation Treatment Effect Pattern Plot analysis of the treatment effect of letrozole versus tamoxifen as measured by 4-year disease-free survival according to overlapping subpopulations defined by percentages of Ki-67 labeling index (LI). The *x*-axis indicates the median percentage of Ki-67 LI for patients in each of the overlapping subpopulations.

More importantly, our analysis provides the first evidence to our knowledge suggesting that Ki-67 LI may have predictive value for the choice of an aromatase inhibitor rather than Tam as adjuvant endocrine therapy among postmenopausal women with endocrine-responsive early breast cancer. Comparison of Let and Tam by Ki-67 LI suggests that Let may be particularly beneficial at higher levels of Ki-67 LI. The hazard of a DFS event was reduced by approximately half in favor of Let for higher levels of Ki-67 LI, which was a treatment effect of greater magnitude than among patients with tumors having low levels of Ki-67 LI. The larger magnitude of benefit for higher Ki-67 LI is in contrast to the lack of such a differential efficacy of Let versus Tam, which we have previously described for HER-2[20] and PgR.[21]

Why should the magnitude of Let superiority over Tam be larger in patients with high tumor Ki-67 LI? One possibility is that among patients with a high tumor proliferation fraction, particularly if associated with overexpression of membrane growth factors (as we show

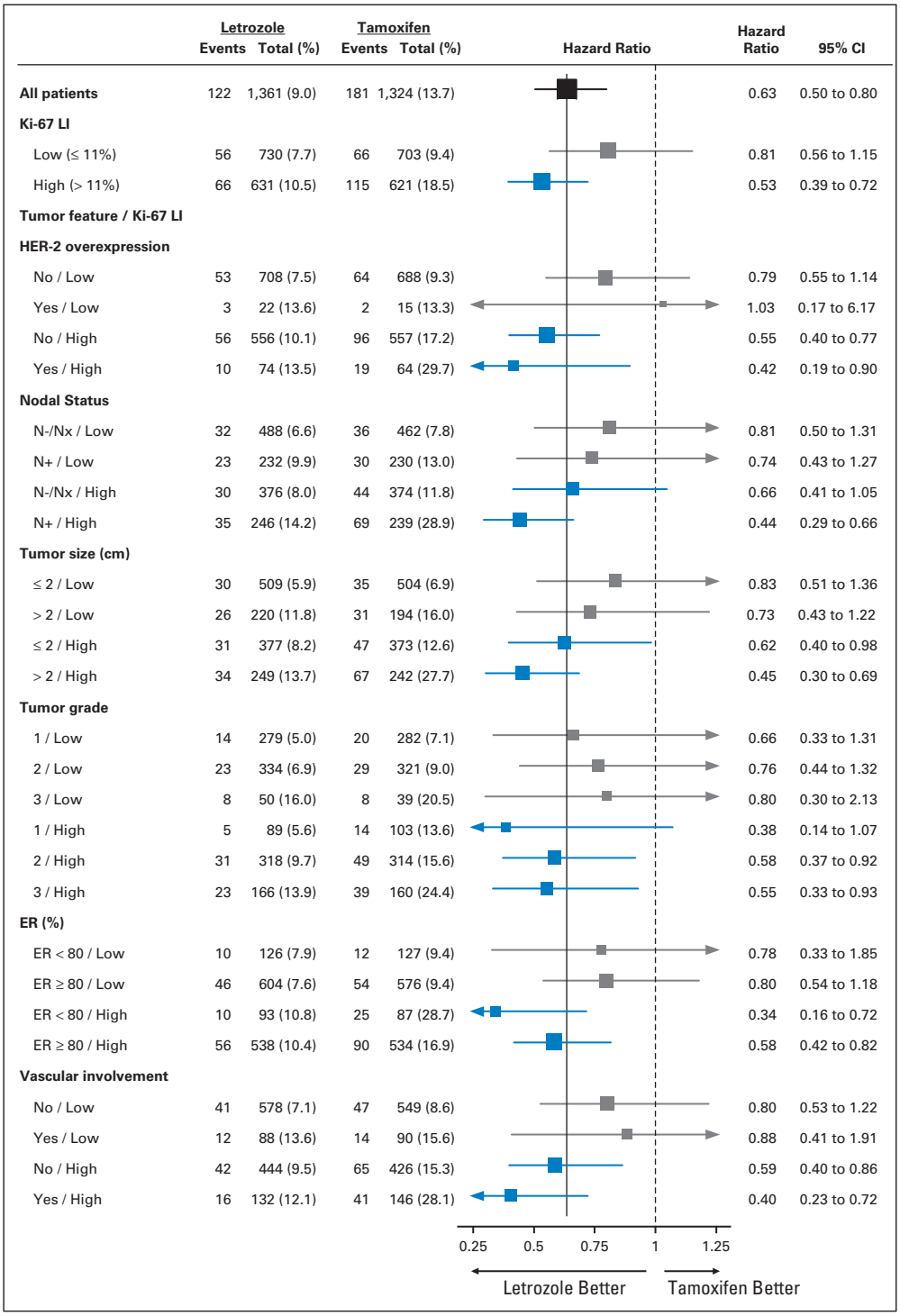

Fig 5. Proportional hazards model results of disease-free survival in subgroups. The size of each box is inversely proportional to the SE of the hazard ratio (HR). The solid vertical line is placed at HR = 0.63, which is the HR estimate for the overall analysis of letrozole compared with tamoxifen in this analytic cohort. HER-2, human epidermal growth factor receptor 2; LI, labeling index; ER, estrogen receptor.

in the present study for HER-2), patients receiving Tam would have higher residual circulating estrogen levels than those receiving an aromatase inhibitor. This residual estrogen, or indeed an agonistic action of Tam itself,[26] may activate membrane ER and combine with high levels of growth factor receptors to worsen prognosis, whereas patients with profound estrogen deprivation induced by an aromatase inhibitor might be protected from tumor cell stimulation through membrane ER.[27] Alternatively, the observed high Ki-67 LI may itself be a reflection of an established growth factor-driven stimulation by residual postmenopausal levels of estrogen through membrane ER crosstalk.[27] In such a scenario, Tam would be less able than profound estrogen depletion to reverse the stimulus to tumor growth.

Particular interest has centered on the choice between initial use of an aromatase inhibitor, as in the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial[28] and the results so far available from the BIG 1-98 trial,[17,18] compared with a policy of switching to an

aromatase inhibitor after 2 or 3 years of adjuvant Tam therapy.[29,30] Pending availability of results from the sequential arms of BIG 1-98, and provided that our observations are confirmed in other studies, it may be that high tumor Ki-67 LI could identify patients in whom the superiority of Let over Tam as initial endocrine therapy is particularly marked.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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