

## Can We Hang Our Hats on One Percent?

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Disclosures of potential conflicts of interest may be found at the end of this article.

The use of markers of proliferation for prognostication in locally advanced breast cancer has long been an area of research interest [1, 2] given the coveted ability to assess treatment effect with dynamic, *in vivo* tumor biomarkers [3]. Although it has previously been shown that higher baseline markers of proliferation are predictive of response to chemotherapy [4–6] and predictive of pathological complete response (pCR) [5, 7], the prognostic value of these of these markers has been controversial and mostly based on retrospective studies [8–12]. Ki-67 has also been included in prognostication models, although these have not been extensively validated, nor have they been widely accepted in clinical practice [13]. Moreover, there has been renewed interest in comparing the Ki-67 levels before and after neoadjuvant chemotherapy (NAC) to assess treatment effect on the tumor microenvironment and to aid in understanding tumor biology, which has also been shown to be prognostic [14, 15]. However, a significant proportion of patients receiving NAC do not achieve pCR, and no multicenter prospective studies evaluating the change in Ki-67 pre- and post-NAC with a correlation to long-term outcomes have been conducted.

In this issue of *The Oncologist*, Crabrera-Galeana and colleagues report their results assessing Ki-67 changes in residual breast cancer after neoadjuvant chemotherapy and correlate the pathology to disease-free survival (DFS) and overall survival (OS). Four hundred thirty-five patients with stage IIA–IIIC breast cancer, who did not achieve pCR, were selected from a database of breast cancer patients treated with NAC at the Instituto Nacional de Cancerologia, in Mexico, between 2007 and 2015. Median follow-up was 27.4 months. All patients included were treated with 4 cycles of an anthracycline and 12 doses of weekly paclitaxel, with trastuzumab added for patients with HER2 positive tumors. Ki-67 was performed on the baseline core biopsy sample and compared with the final surgical specimen. Non-pCR was defined as residual invasive tumor within the breast and/or axilla. The median age of the cohort was 50 years, and patients predominantly had stage III disease (52%), with 68% harboring T3–T4 tumors, 92% with clinically positive nodes and 46% with high-grade tumors. Subtype distribution included 25% luminal A tumors, 45% luminal B tumors, 14% triple-negative tumors, 5% HER2-enriched tumors, and 11% triple-positive tumors. Median Ki-67 before NAC was 20%.

The authors are to be commended for their rigor in specimen analysis; each sample underwent standardized procedures and assays for histological assessment by two highly specialized

breast pathologists. Ki-67 was quantified using a visual scoring system, which included an external control for validation. Residual tumor specimens were then dichotomized into the “decrease” Ki-67 group, defined as a decrease of greater or equal to 1%, and the group with no change or increase, labeled the “non-decrease” group. The “decrease” versus “non-decrease” cohorts were then correlated with DFS and OS.

Of the 435 tumor samples analyzed, 57% of residual tumors had a decrease of Ki-67 positivity of equal or greater than 1%. The median percentage of Ki-67 decreased from 20% before NAC to 10% after NAC. Patients who had a decrease in Ki-67 levels had a longer median DFS as compared with the non-decrease group, 47.6 months (95% confidence interval [CI] 44.1–51.3) versus 38 months (32.7–43.3,  $p < .001$ ), and the 3-year DFS was 82.8% (95% CI 79.3–91.6) as compared with 56.4% (95% CI 45.0–67.8) in the non-decrease group. The results were predominantly driven by the luminal-B subtype, with a DFS of 47 months (95% CI 39.7–47.6) in the decrease group as compared with 36.2 months (29.2–43.3) in the non-decrease group ( $p = .001$ ). No significant difference was identified in the other subtypes, although multivariate analysis revealed that a decrease in Ki-67 was an independent predictor of DFS, with a hazard ratio (HR) of 3.39 (95% CI 1.8–6.37,  $p < .001$ ) in all patients. Furthermore, median OS was 71.2 months (95% CI 68.3–74.2) versus 55.9 months (50.9–60.9,  $p < .0001$ ) in the decrease versus non-decrease group, with a 3-year OS of 97% (95% CI 93.7–100) in the decrease group as compared with 69% (57.6–80.4) in the non-decrease group. Significant differences were once again driven by the luminal B subtype, with an OS of 70.7 months (95% CI 66.7–74.8) in the decrease group as compared with 52.9 months (46.2–59.7) in the non-decrease group ( $p < .0001$ ), with no significant differences identified in the other subtypes. Three-year OS was 95.1% (95% CI 89.6–100) versus 60% (40.2–79.7). Once again, multivariate analysis revealed that a decrease in Ki-67 was a significant independent predictor of OS with an HR of 7.03 (95% CI 2.6–18.7,  $p < .001$ ).

The authors of this study highlight the fact that even a small decrease in Ki-67 after NAC is significantly correlated with better outcomes in terms of DFS and OS. However, the prognostic value of such a small decrease in Ki-67 could potentially signify that the prognostic association represents a continuum, whereby a larger decrease could be predictive of an even greater benefit. Unfortunately, these data cannot be gleaned

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from this study and would require a much larger study population [16]. Nonetheless, a novel contribution of this study is not only that each patient is used as their own control, but that the results have been dichotomized into a binary variable, the decrease versus non-decrease groups, defined as a difference of 1% in the final surgical specimen as compared with the baseline core biopsy. Historically, changes in Ki-67 have had a set change point, with many allocating an arbitrary proportion of change [17], but no study has yet demonstrated that a difference as small as 1% correlates with such significant differences in outcomes. Therefore, one must wonder if the results of this study can truly be reproduced, especially in a multicenter setting where histological analysis may be performed by numerous pathologists who may not be as specialized in oncological care.

Furthermore, review of the results from the preplanned subgroup analysis by subtype suggests that the benefit is predominantly driven by the luminal-B subtype, which represents the largest proportion of patients included in this study. In light of this information, we question once again whether the luminal A patients truly benefit from chemotherapy. This study draws attention to the possible use of Ki-67 in clinical care, allowing an earlier identification of treatment resistance. Such studies are already underway, notably the ALTERNATE study, with a novel design integrating a window of opportunity trial within a neoadjuvant model, using Ki-67 of greater than 10% as an indication of endocrine therapy resistance, resulting in a change in treatment pathways. The POETIC study is another example of the potential use of Ki-67 as a clinical tool to guide treatment, correlating Ki-67 from the surgical specimen to relapse-free survival, following the perioperative use of an aromatase inhibitor.

One of the most notable limitations of the study is undoubtedly the fact that it was performed in a single center. Standardization and reproducibility of Ki-67 remain problematic, with interobserver and interlaboratory variations, despite efforts to uphold a standard of practice globally [18, 19]. The authors did not specify that Ki-67 assessment was done using the International Ki-67 Working Group scoring method, despite their efforts to use a standardized procedure, which perpetuates the difficulties in achieving analytical validity with Ki-67. Although it was necessary for this study to have histological analysis performed by a limited number of pathologists, the applicability in a broader multicenter setting remains unproven, with a potentially large margin of error. Furthermore, the methodology involved between performing Ki-67 on a core biopsy as compared with a surgical specimen is another potential source of error [20]. Given the small percentage of change

considered for the dichotomization of the groups, a potential difference in methodology could be quite significant and alter results, limiting the applicability of this study to the clinical setting. Overcoming this limitation is certainly challenging, and despite many attempts at standardization, the use of Ki-67 has not been widely adopted in the clinical setting. However, the use of automation could be further explored in future research. To this effect, an international Ki-67 working group has found that the precision pathologists achieve while reporting Ki-67 after careful standardization and calibration can be achieved by automated digital image analysis without such special calibration efforts [21]. Future efforts using emerging “deep learning” image analysis methodology have great potential to bring further improvements in the precision of Ki-67 scoring.

Another major limitation stems from the fact that patients who achieved a pCR and who had the best prognosis were excluded from the analysis, as no Ki-67 assay was possible at the time of surgery. Large studies, which viewed Ki-67 changes as a continuous variable as opposed to a dichotomous variable and did sampling during neoadjuvant treatment, could include those who achieve a pCR and may be more valuable in both predicting benefit and defining a wider range of Ki-67 change.

The results of this study are most definitely hypothesis generating and highlight the need for a multicenter, prospective trial, using standardized histological evaluation to support the reproducibility of these findings. Furthermore, although the role of genomic assays has been well established prior to the use of adjuvant chemotherapy, no data are available for their use after neoadjuvant chemotherapy in patients who did not achieve pCR. Alternative and complementary strategies, such as the use of positron emission tomography scans at set time-points in treatment, could also be explored. However, although resources are increasingly limited, the validation of Ki-67 as a prognostic marker for risk of relapse and death would be invaluable and cost-effective. In light of this study, which suggests that the Ki-67 in residual disease correlates with PFS and OS, further consideration should be given to an interim assessment of the Ki-67 to define whether therapy is effective, ultimately guiding treatment decisions for personalized oncological care. It could also allow for the judicious use of adjuvant chemotherapy in the highest-risk patients treated with NAC, given the results of the CREATE-X study [22]. However, caution should be used in applying this information to the clinical setting until robust prospective evidence becomes available.

#### DISCLOSURES

The authors indicated no financial relationships.

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#### Editor's Note:

See the related article, "Ki67 Changes Identify Worse Outcomes in Residual Breast Cancer Tumors After Neoadjuvant Chemotherapy," by Oscar Arrieta et al., on page 670 of this issue.