Article

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Simple Summary

Doctors caring for patients with early-stage breast cancer often rely on tests that estimate how actively cancer cells are growing to help decide whether chemotherapy is needed. One commonly used test examines tumor samples under a microscope, while another analyzes the activity of many genes within the cancer. These tests are sometimes thought to provide similar information, but how well

they agree in everyday clinical practice is unclear. In this study, we examined data from a large group of women in Greece with hormone receptor–positive, HER2-negative early breast cancer who underwent gene-based tumor testing. We found that the simple laboratory marker and the gene-based test often gave different results for individual patients. This highlights the importance of genomic testing for personalized treatment decisions and supports current medical guidance against using simpler tests as substitutes.

Abstract

Background/Objectives: Ki-67 is widely used as an immunohistochemical marker of tumor proliferation in hormone receptor–positive (HR-positive), HER2-negative breast cancer, but its interpretation is limited by variability and uncertain concordance with genomic assays. The Oncotype DX® Recurrence Score (RS) is a validated multigene assay with established prognostic and predictive utility. This study evaluated the relationship between Ki-67 and RS in clinical practice. **Methods:** We retrospectively analyzed women in Greece with early-stage estrogen receptor–positive, HER2-negative breast cancer without distant metastasis (pM0) who underwent Oncotype DX testing between 2020 and 2023. Eligible patients were node negative or postmenopausal with node positive disease. RS was categorized as low (0–25) or high (>25). Ki-67 was assessed using binary (<20% vs ≥20%) and three-tier (≤5%, >5%–<30%, ≥30%) classifications. Associations were analyzed using correlation, concordance, and nonparametric methods. **Results:** Among 2,967 patients, the median RS was 16, with similar distributions across nodal subgroups. Ki-67 and RS demonstrated a moderate positive correlation as continuous variables ($r = 0.42$, $p < 0.001$). After stratification, associations with RS were observed only in tumors with high Ki-67 expression, whereas no correlation was detected in low or intermediate groups. RS distributions differed significantly across Ki-67 strata. Overall concordance between Ki-67-based proliferation categories and RS-based genomic risk was 56.2%, with discordant cases in both directions. **Conclusions:** Ki-67 shows a moderate association with Oncotype DX RS, but substantial discordance indicates it should not substitute genomic testing in HR-positive/HER2-negative early breast cancer.

Keywords: Ki-67; Oncotype DX; recurrence score; hormone receptor–positive breast cancer; HER2-negative breast cancer; tumor proliferation; genomic assays; adjuvant therapy decision-making; early-stage breast cancer

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1. Introduction

Breast cancer is the most frequently diagnosed malignancy worldwide and a leading cause of cancer related mortality[1]. Owing to its biological heterogeneity, accurate tumor characterization is essential for prognostic assessment and therapeutic decision making.

In routine clinical practice, this characterization relies on a standardized panel of biomarkers, including mainly estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), along with the Ki 67 proliferation index, which together inform tumor subtype assignment, prognosis, and selection of systemic therapies [2–4].

Among these biomarkers, Ki 67 is a nuclear protein expressed during active phases of the cell cycle and serves as a marker of cellular proliferation. In hormone receptor–positive, HER2 negative breast cancer, higher Ki 67 expression has been consistently associated with increased tumor aggressiveness, higher histologic grade, and worse clinical outcomes [5–7].

Ki 67 immunohistochemical expression has long been used as a surrogate marker of tumor proliferative activity in breast cancer; however, the optimal cut off values for clinical interpretation have evolved over time. Early prognostic studies in the late 1990s and early 2000s commonly applied low Ki 67 thresholds around 10%, showing that increased proliferative activity was associated with inferior disease free and overall survival, albeit with substantial inter study variability due to differences in assessment methodology and scoring [8].

The 2011 St Gallen Consensus proposed a Ki 67 cut off of 14% to distinguish luminal A–like from luminal B–like tumors when immunohistochemistry was used as a surrogate for intrinsic molecular subtyping, based on correlations with gene expression profiling [9]. However, concerns regarding limited reproducibility and inter laboratory variability prompted reassessment in the 2013 St Gallen Consensus, which suggested that a higher threshold of approximately 20% might better identify tumors with increased proliferative activity [10]. Subsequent studies supported this shift, demonstrating improved concordance between Ki 67 ≥20% and genomic risk signatures, leading to its broad adoption as a marker of high proliferation in hormone receptor–positive breast cancer and its incorporation into clinical practice and trial designs [11,12].

The clinical relevance of a Ki 67 cut off ≥20% was further highlighted by the monarchE trial, in which this threshold, together with other clinicopathologic criteria, was used to classify patients with 1–3 positive axillary lymph nodes (N1) as having high risk disease [13] ; although subsequent analyses demonstrated Ki 67 to be prognostic but not predictive of abemaciclib benefit, leading to removal of this criterion from mandatory eligibility requirements [14,15].

More recently, recognition of Ki 67 as a continuous biological variable, rather than a dichotomous marker, has shaped contemporary guideline recommendations. The International Ki 67 in Breast Cancer Working Group (IKWG) proposed a three tier framework to improve interpretability while accounting for analytic variability: very low values (≤5%), very high values (≥30%), and an intermediate range (5–30%) [16]. Within this framework, Ki 67 provides meaningful prognostic information at the extremes, whereas intermediate values should not guide treatment decisions in isolation and require integration with clinicopathologic factors and/or genomic assays.

Despite its widespread use, the clinical interpretation of Ki 67 remains challenging due to substantial inter observer and inter laboratory variability, differences in pre analytical and analytical handling, heterogeneity in scoring methodologies, and the lack of universally accepted cut off values [16–22]. These limitations are consistently acknowledged across all major international guidelines and consensus statements, including St Gallen, ESMO, ASCO, and the International Ki-67 in Breast Cancer Working Group [11,16,23,24].

Although Ki 67 is a well established prognostic marker in ER positive/HER2 negative breast cancer, evidence supporting its predictive ability for chemotherapy benefit remains inconsistent and insufficient for clinical decision making.

In a systematic review and meta-analysis of 53 studies including 10,848 patients, pre-treatment Ki-67 was associated with higher pathological response rates to neoadjuvant chemotherapy in breast cancer, regardless of HR-positive, HER2-positive, or triple-negative disease subtype [25]. Similar trends have been reported in additional studies across all breast cancer molecular subtypes; however, the use of heterogeneous Ki 67 cut off values across these studies has raised further questions regarding the robustness and clinical applicability of Ki 67 [26–29].

However, several studies have failed to demonstrate a correlation between Ki 67 and pathological complete response [30,31], and while pCR is strongly associated with favorable long term outcomes in HER2 positive and triple negative breast cancer, this relationship is less evident in HR+/HER2− breast cancer, where pCR is less common.

In prospective–retrospective analyses of the randomized IBCSG VIII and IX trials, in which patients were assigned to chemotherapy plus endocrine therapy versus endocrine therapy alone, a high Ki 67 labeling index was associated with worse disease free survival across all treatment arms. Importantly, no interaction between Ki 67 expression and treatment assignment was observed, indicating that the effect of treatment on disease free survival was independent of Ki 67 status and supporting a prognostic rather than predictive role for Ki 67 [32].

Given the insufficient evidence to support Ki 67 as a predictive marker for adjuvant chemotherapy benefit, International Ki 67 in Breast Cancer Working Group and ASCO have concluded that Ki 67 has established clinical validity as a prognostic biomarker but should not be used alone to guide chemotherapy decisions in ER positive/HER2 negative breast cancer [16,23].

These limitations have prompted increasing interest in multigene expression assays that offer standardized and reproducible assessments of tumor biology.

Oncotype DX is a validated multigene expression assay that characterizes tumor biology by analyzing the expression of 21 genes related to proliferation, hormone receptor signaling, HER2 signaling, invasion, and other biologic processes, generating a Breast Recurrence Score® (RS) ranging from 0 to 100. Ki 67 (MKI67) constitutes a key component of the proliferation gene module, which contributes substantially—but not exclusively—to the overall Recurrence Score. In patients with early-stage, hormone receptor–positive, HER2-negative breast cancer, the assay has been extensively validated in both retrospective and prospective studies as a prognostic marker of recurrence risk and a predictive tool for chemotherapy benefit, including in patients with node-negative disease and those with up to three positive axillary lymph nodes [33–40]. On the basis of results from pivotal prospective trials TAILORx and RxPONDER, the Oncotype DX Recurrence Score has been integrated into international treatment algorithms to guide adjuvant chemotherapy decision making [33,35]. According to NCCN guidelines, in postmenopausal patients or those aged ≥50 years with node negative (N0) or limited node positive (N1, 1–3 positive nodes) disease, a Recurrence Score (RS) of 0–25 supports omission of adjuvant chemotherapy, whereas scores >25 identify patients for whom chemotherapy is recommended. In premenopausal patients with node negative disease, no benefit has been observed from the addition of chemotherapy to standard endocrine therapy for RS 0–15, while RS 16–25 has been associated with a trend towards absolute benefit from chemotherapy addition to standard endocrine therapy, as demonstrated in pre specified subgroup analyses of the TAILORx trial, and RS ≥26 indicate a clear recommendation for adjuvant chemotherapy [41].

Given that Ki 67 and the Recurrence Score both capture aspects of tumor proliferative activity, evaluating their relationship may provide insight into the concordance and potential added value of these biomarkers.

In a systematic review and meta-analysis encompassing 18 studies with sample sizes ranging from 53 to 4,695 (median sample size, 106), Ki 67 demonstrated a positive association with the Oncotype DX Recurrence Score in hormone receptor positive, HER2 negative early breast cancer, with the exception of two studies [42]. However, pronounced inter study heterogeneity, together with substantial methodological variability, including the use of heterogeneous cut off values for both Ki 67 expression and Recurrence Score risk categories—limited its ability to reliably predict Recurrence Score categories, reinforcing that Ki 67 cannot substitute for multigene assays. Consistent with this meta-analysis, additional studies in the literature have yielded conflicting results regarding the relationship between Ki 67 and the Oncotype DX Recurrence Score, with some reporting a positive association [43–47] while others failed to demonstrate a significant correlation [48–50], further underscoring the limited reliability of Ki 67 for Recurrence Score prediction. Of interest, one study reported that although no significant correlation between Ki 67 and Recurrence Score was observed in the overall study population, a significant association emerged within a predefined subgroup of tumors with high Ki 67 expression (≥30%), suggesting that the relationship between Ki 67 and genomic risk may be restricted to highly proliferative tumors [50]. Beyond issues of statistical correlation, several studies have emphasized that Ki 67 and the Oncotype DX Recurrence Score may assign discordant risk categories at the individual patient level, reflecting fundamental differences in how single marker proliferation and multigene genomic profiling capture tumor biology and inform clinical risk stratification [47,51,52].

The objective of this study is to examine the association between the Ki 67 proliferative index and the Oncotype DX Recurrence Score, and to assess its potential value in predicting the Recurrence Score.

2. Materials and Methods

Study Design and Data Collection

This was a retrospective, multicenter cohort study including women diagnosed with early stage, estrogen receptor (ER)–positive, HER2 negative invasive breast cancer who underwent Oncotype DX® Breast Recurrence Score (RS) testing between 2020 and 2023 across multiple institutions in Greece. Clinical and pathological variables, including age, nodal status, tumor size, histologic grade, Ki 67 proliferation index, and RS results, were collected through comprehensive review of medical records.

Definition of Variables

The Oncotype DX Recurrence Score was analyzed both as a continuous variable and as a categorical variable, using clinically relevant thresholds. Patients were classified into low genomic risk (RS 0–25) and high genomic risk (RS >25), in accordance with contemporary clinical trial evidence and guideline recommendations.

Ki 67 expression was evaluated using two approaches:

1. A binary classification (<20% vs ≥20%).
2. A three tier classification defining low (≤5%), intermediate (>5% and <30%), and high (≥30%) Ki 67 expression, as proposed by the International Ki 67 in Breast Cancer Working Group.

Nodal status was categorized as node negative (N0), micrometastatic nodal involvement (N1mic), or node positive disease (N1). Histologic grade was assessed according to standard pathology criteria and analyzed as grades 1, 2, or 3.

Statistical Analysis

Descriptive statistics were used to summarize baseline clinical and pathological characteristics. Continuous variables were reported as medians with ranges, and categorical variables as frequencies and percentages. Data processing and visualization were performed using both Python (version 3.12.2) and R (version 4.5.3) . In Python, pandas was used for data handling, while matplotlib and seaborn were used to generate heatmaps, scatter plots, and distribution plots. In parallel, additional analyses and visualizations were performed in R using ggplot2 and ggpubr, including violin plots and concordance analyses. Ki-67 was analyzed using predefined categorical groupings (<20% vs. ≥20% and ≤5%, >5–<30%, ≥30%), and RS distributions were assessed with non-parametric tests (Wilcoxon rank-sum and Kruskal–Wallis), with results reported as p-values.

Distribution Analyses

The distribution of Recurrence Score categories was assessed across nodal subgroups and Ki 67 expression categories. Comparisons of RS distributions across Ki 67 groups were performed using non parametric statistical tests, given the non normal distribution of RS values. These included the Wilcoxon rank sum test for two group comparisons and the Kruskal–Wallis test for comparisons across more than two groups. Results were visualized using boxplots and distribution plots.

Correlation Analyses

the association between ki 67 and recurrence score was evaluated using pearson’s correlation coefficient, treating both variables as continuous measures. correlation analyses were performed in the overall cohort and after stratification by ki 67 expression categories (binary and three tier classifications) to assess whether the strength of association differed across proliferative subgroups. scatter plots were generated to illustrate correlation patterns.

Categorical Association and Concordance Analysis

A categorical association analysis was conducted by cross classifying Ki 67 expression (binary classification: <20% vs ≥20%) with RS risk categories (0–25 vs >25). Concordance between Ki 67–defined proliferation categories and RS based genomic risk classification was calculated as the proportion of patients classified concordantly. Discordant classifications were quantified and reported descriptively. Results were displayed using contingency tables and heatmaps.

Combined Analyses by Histologic Grade and Ki 67

Median Recurrence Scores were evaluated across combinations of histologic grade and Ki 67 expression categories. A heatmap was generated to visualize RS patterns across grade (1–3) and Ki 67 three tier groups, enabling assessment of the combined impact of tumor differentiation and proliferative activity on genomic risk.

3. Results

From 2020 to 2023, a total of 2,967 patients in Greece with newly diagnosed, invasive, early-stage breast cancer underwent Oncotype DX Breast Recurrence Score testing across several institutions. Eligible patients had ER positive, HER2 negative disease and were either node negative (pN0) or postmenopausal with node positive disease (pN1), with no evidence of distant metastasis (pM0). Baseline clinical and pathological characteristics of the study population are summarized in Table 1.

Table 1. Clinical characteristics of the study population.

Characteristic	
Median age (range)-yr	56 (21-85)
Age — no. (%)	
≤50	990(33.4)
>50	1977 (66.6)
Nodal Staus — no. (%)	
N0	2636 (88.9)
N1mic	84 (2.8)
N1- 1 positive lymph node	171 (5.8)
N1- 2 positive lymph nodes	60 (2.0)
N1- 3 positive lymph nodes	16 (0.5)
Median tumor size (range)-cm	1.5 (0.2-10.2)
Tumor size — no. (%)	
T1a (≤0.5cm)	61 (2.1)
T1b (>0.5cm and ≤ 1cm)	646 (21.8)
T1c (>1cm and ≤ 2cm)	1551 (52.2)
T2 (>2cm and ≤ 5cm	678 (22.9)
T3 (>5cm)	31 (1)
Histologic grade at diagnosis — no. (%)	
1	355 (12.0)
2	1988 (67.0)
3	624 (21.0)
Median Ki67 (%)	20
Ki67 — no. (%)	
Binary classification	
<20 %	1378 (46.4)
≥20 %	1589 (53.6)
Three-tier classification	
≤5 %	203 (6.8)

>5 % & <30 %	2030 (68.4)
≥30 %	734 (24.8)

In the overall study population (n = 2,967), the median RS was 16; 2,522 patients (85.0%) were categorized in the RS 0–25 group, whereas 445 patients (15.0%) had RS >25. (Available data in Supplemental File 1)

Distribution of Recurrence Score and Nodal Status

Among node negative patients (N0; n = 2,636), 2,232 patients (84.7%) had an RS of 0–25, while 404 patients (15.3%) had an RS >25. In patients with micrometastatic nodal disease (N1mic; n = 84), 75 patients (89.3%) were classified with RS 0–25 and 9 patients (10.7%) with RS >25. Similarly, among patients with node positive disease (N1; n = 247), 215 patients (87.0%) had RS 0–25 and 32 patients (13.0%) had RS >25.

The distribution of Oncotype DX Recurrence Scores was similar across nodal subgroups, with no appreciable differences observed between patients with node negative, micrometastatic, or node positive disease (Figure 1).

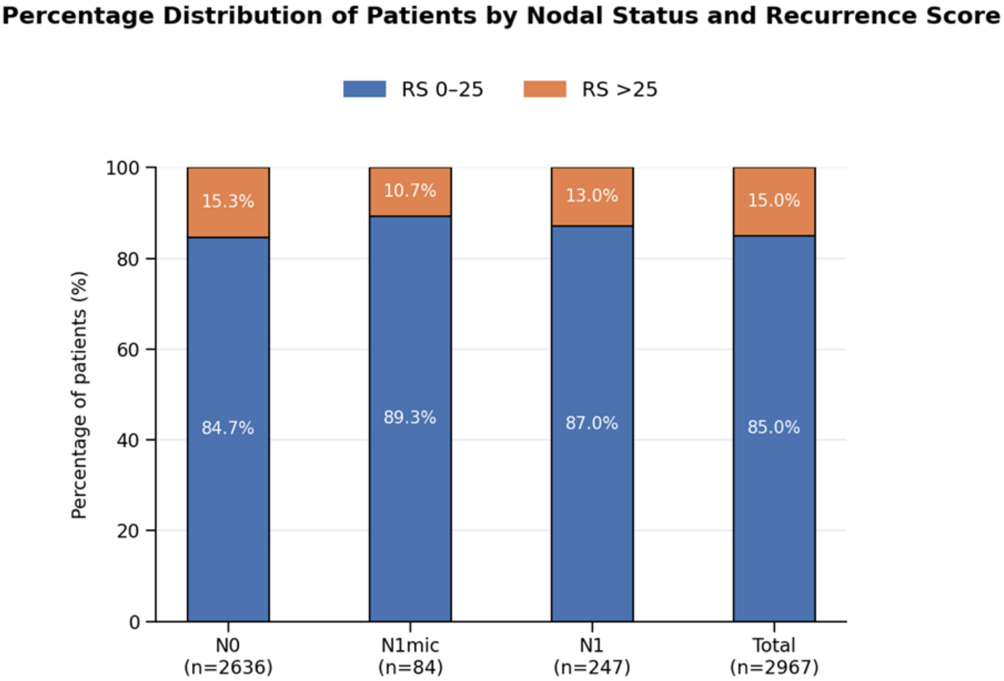

Figure 1. Percentage distribution of patients with low (RS 0–25) and high (RS >25) genomic risk across nodal subgroups (N0, N1mic, N1) and the total population. Stacked bars show the proportion of patients within each group. Sample sizes are shown on the *x*-axis. RS denotes Recurrence Score.

Correlation Between Ki 67 Expression and Recurrence Score

Analysis of Ki 67 and Recurrence Score (RS) as continuous variables demonstrated a moderate positive correlation in the overall study population (Pearson's r = 0.42, p < 0.001). After stratification by Ki 67 expression using either a binary cut off (<20% vs ≥20%) or a three tier classification (≤5%, >5% to <30%, and ≥30%), a moderate positive linear correlation with RS was observed exclusively among tumors with high Ki 67 expression (≥20% or ≥30%, depending on the cut off applied), whereas no significant correlation was identified in tumors with low or intermediate Ki 67 expression (Figure 2).

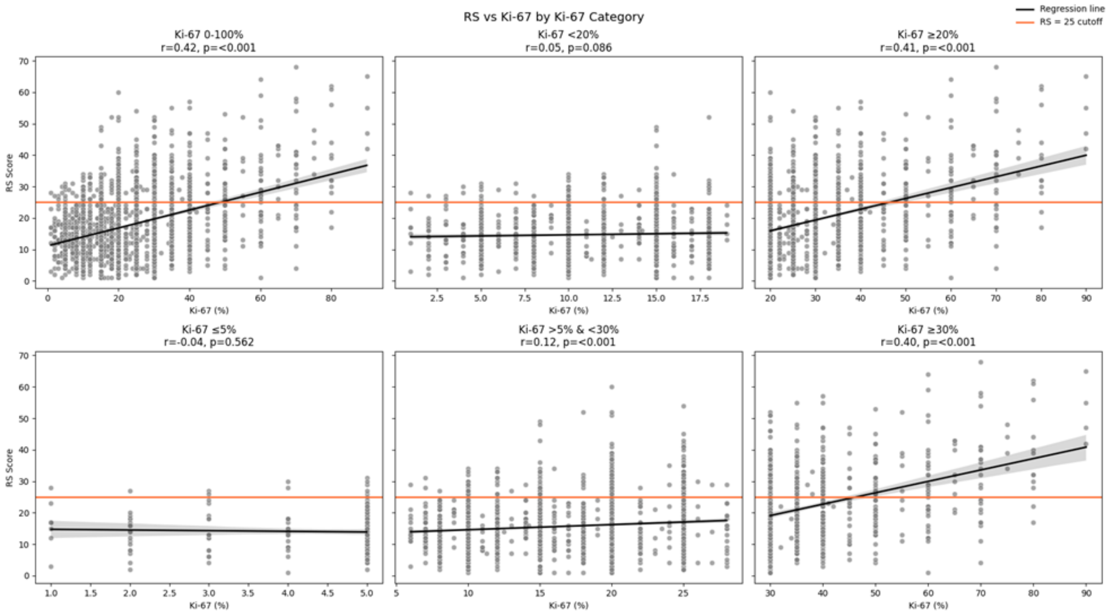

Figure 2. Relationship between Ki67 expression and Oncotype DX Recurrence Score (RS) in the overall cohort and across Ki67-defined subgroups. Scatter plots illustrate RS values plotted against Ki67 (%) for the entire population and within predefined Ki67 categories (<20%, ≥20%, ≤5%, >5%–<30%, and ≥30%). Linear regression lines (black) and the RS cutoff of 25 (red line) are shown. Pearson correlation coefficients (r) and p-values are indicated in each panel.

Distribution of Recurrence Score Across Ki 67 Categories

Comparison of oncotype dx recurrence score distributions across ki 67 expression groups revealed a statistically significant difference in rs distribution, with patients exhibiting higher ki 67 expression demonstrating a shift toward higher recurrence scores. This finding was consistently observed using both the binary (<20% vs ≥20%) and three tier (≤5%, >5%–<30%, ≥30%) ki 67 classification systems (figures 3-4).

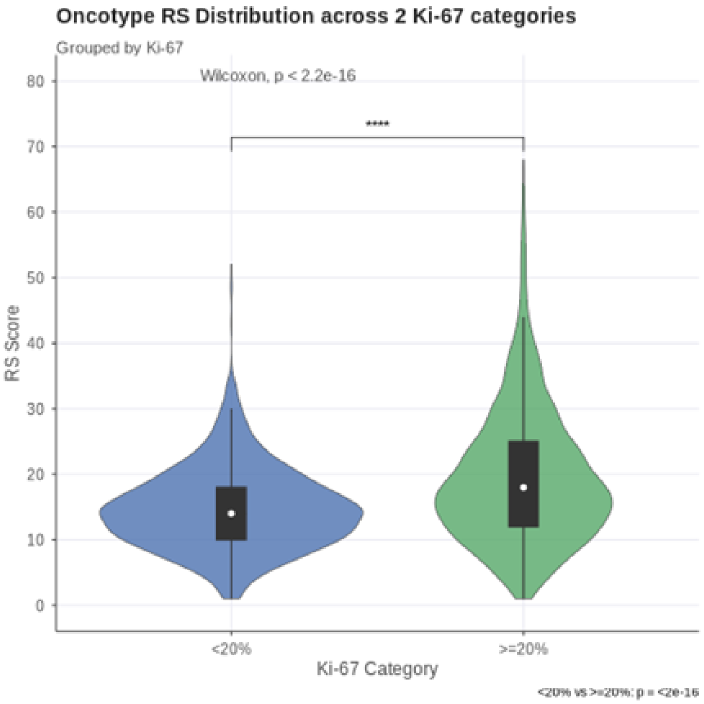

Figure 3. Distribution of oncotype dx recurrence scores (rs) stratified by ki67 expression (<20% vs ≥20%). Violin plots depict the distribution of rs values, with embedded boxplots showing the median and interquartile range. The white dot denotes the median.

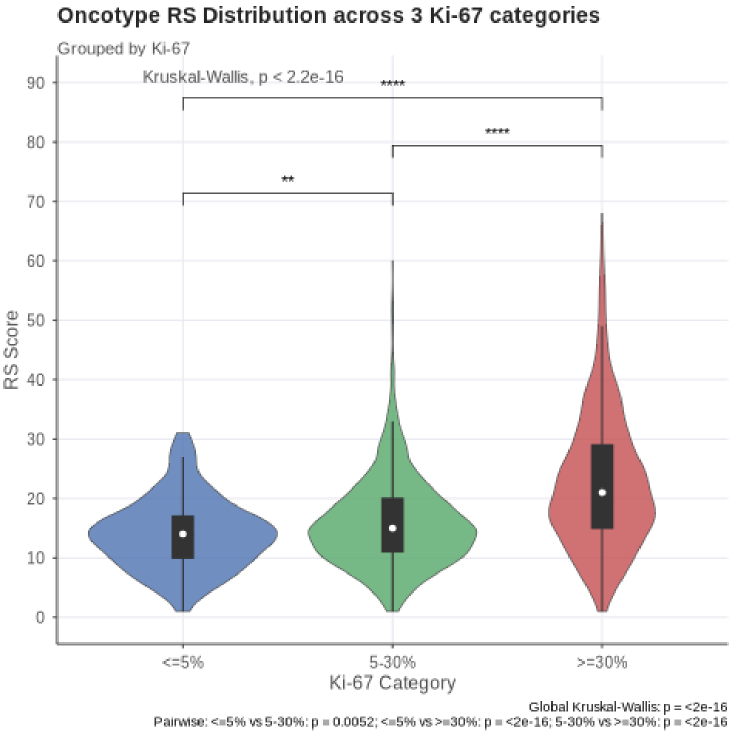

Figure 4. Distribution of oncotype dx recurrence scores (rs) across three-tier ki67 expression categories (≤5%, >5%–<30%, ≥30%). Violin plots display the distribution of rs values, with embedded boxplots showing the median and interquartile range. The white dot denotes the median.

Categorical Association Between Ki-67 and Genomic Risk

In addition to continuous analyses, a categorical association analysis was conducted by stratifying patients according to Ki 67 expression (<20% vs ≥20%) and Oncotype DX Recurrence Score (RS 0–25 vs >25) to assess concordance between Ki 67 defined proliferation categories and Recurrence Score–based genomic risk classification. Overall, concordance between Ki 67 status and genomic risk category was observed in 56.2% of cases. Discordant classifications were also identified: 5.6% of patients with low Ki 67 expression (<20%) had a high Recurrence Score (>25), whereas 76.9% of patients with high Ki 67 expression (≥20%) were classified as low genomic risk (RS 0–25) (Figure 5).

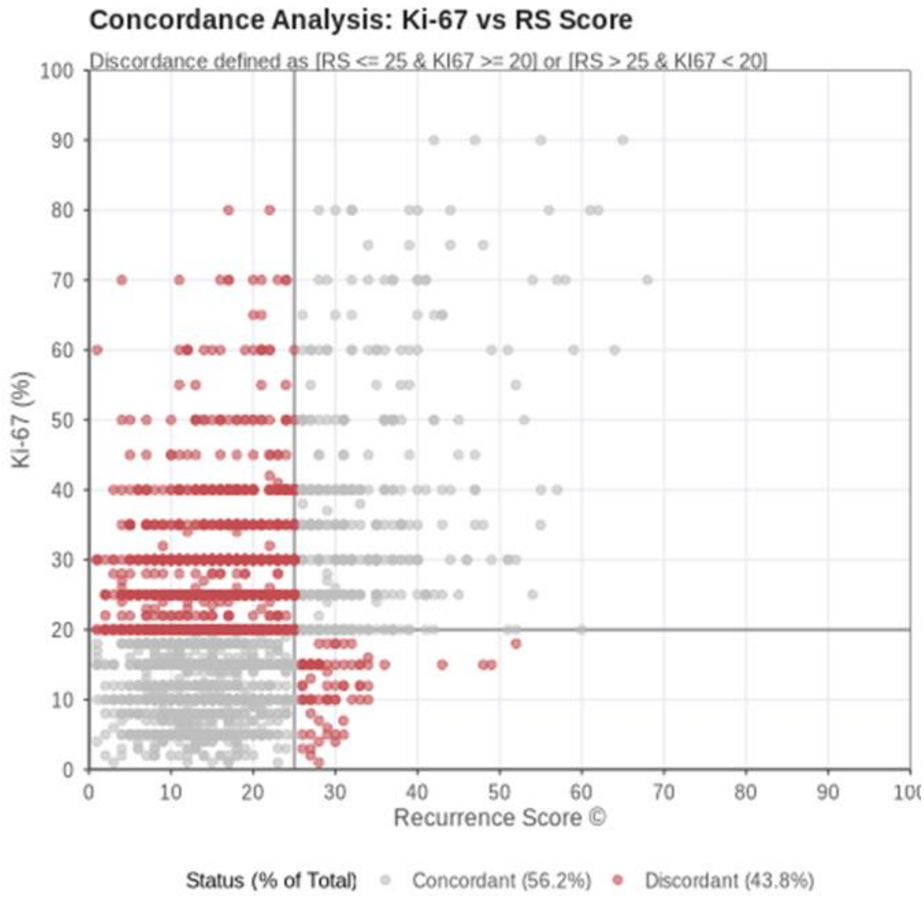

Figure 5. Concordance between Ki67 expression and Oncotype DX Recurrence Score (RS) at the individual patient level. Each point represents a patient plotted by Ki67 (%) and RS. Horizontal and vertical lines indicate clinically relevant thresholds (Ki67 20% and RS 25), dividing patients into concordant and discordant categories. Concordant cases (grey) represent agreement between Ki67-defined proliferation status and RS-based genomic risk, whereas discordant cases (red) represent mismatched classifications (RS ≤25 with Ki67 ≥20% or RS >25 with Ki67 <20%).

Based on the three tier Ki 67 classification, high RS (>25) was observed in 5.4% of patients with low Ki 67 levels, 9.3% of those with intermediate Ki 67 levels, and 33.5% of those with high Ki 67 levels (Table 2).

Table 2. Distribution of Oncotype DX Recurrence Score categories across three tier Ki 67 expression groups.

	N(%)
Ki 67 ≤5%	**203**
-RS 0-25	192 (94.6)
-RS >25	11 (5.4)
Ki 67 >5% and <30%	**2030**
-RS 0-25	1842 (90.7)
-RS >25	188 (9.3)
Ki 67 ≥30%	**734**
-RS 0-25	488 (66.5)
-RS >25	246 (33.5)

Recurrence Score Distribution Across Histologic Grade and Ki-67

Analysis of median Oncotype DX Recurrence Score (RS) according to tumor grade and Ki 67 category is presented in Figure 6. Across all histologic grades, median RS values increased with higher Ki 67 expression. A similar trend of increasing RS was observed with increasing tumor grade within each Ki 67 category.

In grade 1 tumors, the median RS increased from 13.0 in the Ki 67 ≤5% category to 13.5 in the >5%–<30% category and to 16.0 in the Ki 67 ≥30% category. In grade 2 tumors, corresponding median RS values were 14.0, 15.0, and 18.0, respectively. The highest RS values were observed in grade 3 tumors, with a median RS of 11.5 in the Ki 67 ≤5% group, increasing to 18.0 in the >5%–<30% group and to 23.0 in the Ki 67 ≥30% group.

Overall, higher Ki 67 expression and higher tumor grade were both associated with higher median Recurrence Scores, with the combination of grade 3 tumors and Ki 67 ≥30% demonstrating the highest median RS values.

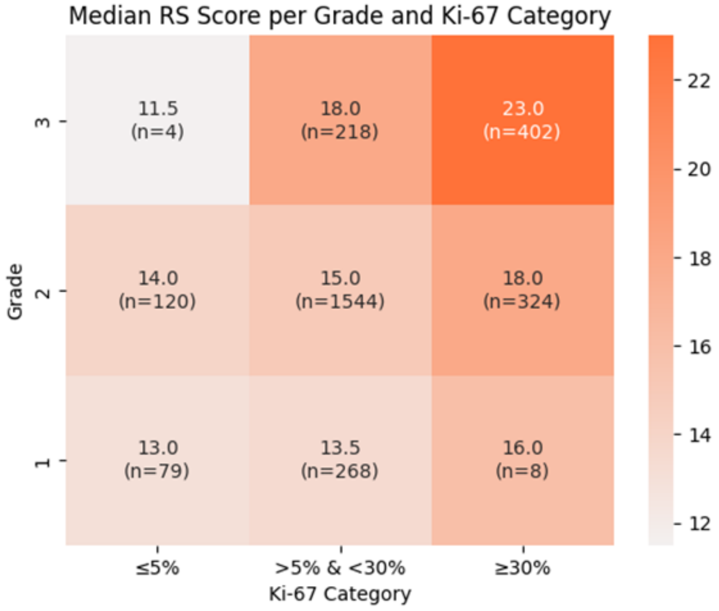

Figure 6. Heatmap illustrating median Oncotype DX Recurrence Score (RS) according to combined histologic grade (grades 1–3) and Ki67 expression categories (≤5%, >5%–<30%, ≥30%). Each cell shows the median RS and the number of patients (n).

4. Discussion

In this real-world retrospective cohort, we identified a moderate overall correlation between Ki 67 and the Oncotype DX Recurrence Score (r = 0.42), in line with prior studies reporting correlation coefficients in the range of 0.3–0.4 [45,53,54]. Importantly, stratification by clinically relevant Ki 67 cut offs revealed that this association was heterogeneous and largely contingent on higher Ki 67 expression levels. Specifically, no meaningful correlation was observed among tumors with low proliferative activity (Ki 67 <20%, Ki 67 ≤5%, or Ki 67 >5% and <30%), whereas a moderate association emerged in tumors with elevated Ki 67 expression (Ki 67 ≥20% or ≥30%). Similar patterns, in which the association between Ki 67 and genomic risk becomes apparent predominantly at higher proliferative levels, have been reported in other studies [50].

These findings suggest that the observed global correlation is predominantly driven by highly proliferative tumors, while low proliferation tumors exhibit biological heterogeneity that is not adequately captured by Ki 67 alone.

Concordance Analysis: Limited Predictive Performance of Ki-67

The broad dispersion of Recurrence Score (RS) values observed across all Ki-67 strata underscores substantial underlying biological heterogeneity within each Ki-67 category. Using a clinically relevant Ki-67 cut-off of 20%, concordance between Ki-67–defined proliferative status and RS-based genomic risk classification was limited to 56.2%, indicating that Ki-67 accurately reflected genomic risk in just over half of cases. Such a degree of agreement is insufficient to support clinical decision-making and aligns with prior reports highlighting the limited reliability of Ki-67 alone in predicting Recurrence Score categories, with marked discordance across the full range of Ki-67 expression [52]. Notably, 76.9% of tumors with elevated Ki-67 (≥20%) were classified as low genomic risk (RS ≤25), while a smaller but clinically meaningful proportion of tumors with low Ki-67 exhibited high Recurrence Scores (5.6%), illustrating the potential for both overtreatment and undertreatment if Ki-67 were used as a surrogate for genomic testing.

Additional insight was provided by analysis using the three-tier Ki-67 classification, which revealed that most tumors (68.4%) clustered within the intermediate Ki-67 range, a category for which prognostic discrimination is limited according to International Ki-67 in Breast Cancer Working Group recommendations16. Within the low Ki-67 group (≤5%), only a small fraction of tumors (5.4%) was associated with high Recurrence Scores, whereas in the high Ki-67 group (≥30%), most patients (66.5%) demonstrated low genomic risk (RS 0–25). Collectively, these findings reinforce that Ki-67 and multigene assays capture overlapping but non-equivalent dimensions of tumor biology and should not be regarded as interchangeable tools for individualized risk stratification.

Biological Interpretation: Proliferation Versus Tumor Biology

The observed discordance can be explained by fundamental biological differences between Ki-67 and the Recurrence Score (RS). Ki-67 represents a single-marker assessment of cellular proliferation, whereas RS integrates the expression of 21 genes involved not only in proliferation but also in estrogen signaling, invasion, and other key tumor biology pathways[36]. This distinction is particularly evident in tumors with low Ki-67 expression, where the absence of correlation suggests that non-proliferative biological processes contribute substantially to genomic risk. Conversely, in highly proliferative tumors, Ki-67 contributes more prominently to RS, accounting for the moderate correlation observed in this subgroup. Nonetheless, even within highly proliferative tumors, the broad range of RS values indicates that proliferation alone is insufficient to fully define tumor behavior. In addition, well-recognized inter-laboratory variability and lack of analytical standardization further limit the reliability of Ki-67 as a predictive biomarker [16–22]

High Clinical Risk Does Not Equate to High Genomic Risk

One of the most clinically relevant findings of this study is that patients with traditionally high-risk clinicopathologic features—specifically high Ki-67 (≥30%) and high histologic grade (G3)—frequently exhibited RS values below the chemotherapy treatment threshold. These results reinforce the concept that clinicopathologic and genomic risk are not interchangeable. Similar observations have been reported in real-world cohorts, where tumors with aggressive histopathologic characteristics may nonetheless demonstrate low genomic risk [55–57]. Clinically, this suggests that a subset of patients who would historically be considered for chemotherapy based on elevated Ki-67 or tumor grade may safely forgo cytotoxic treatment when therapy is guided by genomic profiling.

Consistency Across Nodal Subgroups

We further observed that RS distributions were comparable across nodal subgroups (N0, N1mic, N1), supporting the notion that RS primarily reflects intrinsic tumor biology rather than anatomic disease burden and suggesting that genomic risk, as assessed by RS, is largely independent of nodal involvement. This finding is consistent with evidence from large prospective trials, including

TAILORx and RxPONDER, which demonstrated that the prognostic and predictive value of RS extends across nodal categories, particularly in postmenopausal patients[33,35].

Clinical Implications

Taken together, these findings have important implications for clinical practice. Although Ki-67 remains a useful prognostic marker and provides insight into tumor proliferative activity, it cannot reliably predict genomic risk as defined by RS. Consequently, reliance on Ki-67 alone to guide adjuvant chemotherapy decisions may lead to inappropriate treatment selection. Our results support the continued use of validated multigene assays in patients with HR-positive, HER2-negative early breast cancer, particularly in scenarios where clinicopathologic features suggest high risk but treatment decisions remain uncertain. This consideration is especially relevant in healthcare settings where access to genomic testing may be limited, and surrogate biomarkers are sometimes contemplated.

Limitations

This study has several limitations. First, Ki-67 assessment was not centrally reviewed, introducing potential inter-laboratory variability—a known limitation of Ki-67 interpretation. Second, the retrospective design carries inherent risk of selection bias. Third, certain biologically discordant subgroups (e.g., G1 tumors with high Ki-67 or G3 tumors with low Ki-67) were underrepresented, reflecting real-world distributions but limiting the robustness of subgroup analyses.

5. Conclusions

In this large real-world cohort, Ki-67 demonstrated a moderate correlation with RS, driven predominantly by tumors with high proliferative activity. However, the substantial discordance observed across clinically relevant Ki-67 thresholds indicates that Ki-67 cannot serve as a surrogate for genomic testing. These findings reinforce the critical role of multigene assays in accurately stratifying recurrence risk and guiding adjuvant treatment decisions in early-stage HR-positive/HER2-negative breast cancer.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of EXEM (Hellenic Society of Breast Surgery).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data derived from patients' anonymized medical records are available in Supplemental File 1.

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Abbreviations

The following abbreviations are used in this manuscript:

Abbreviation	Full Name
Ki-67	Ki-67 proliferation index (Ki-67 nuclear antigen)
ER	Estrogen receptor
PR	Progesterone receptor
HER2	Human epidermal growth factor receptor 2
HR	Hormone receptor
RS	Recurrence Score

Oncotype DX	Oncotype DX Breast Recurrence Score assay
MKI67	Marker of proliferation Ki-67 gene
pM0	Pathologic absence of distant metastasis
pN0	Pathologic node-negative disease
pN1	Pathologic node-positive disease
N0	Node-negative disease
N1	Node-positive disease
N1mic	Micrometastatic nodal involvement
G1	Histologic grade 1
G2	Histologic grade 2
G3	Histologic grade 3
pCR	Pathological complete response
IKWG	International Ki-67 in Breast Cancer Working Group
ESMO	European Society for Medical Oncology
ASCO	American Society of Clinical Oncology
NCCN	National Comprehensive Cancer Network
IBCSG	International Breast Cancer Study Group
TAILORx	Trial Assigning Individualized Options for Treatment (Rx)
RxPONDER	Rx for Positive Node, Endocrine Responsive Breast Cancer
monarchE	monarchE clinical trial
r	Pearson correlation coefficient
p	p-value

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