

The Prognostic Significance of the NOLUS Score in Patients with Metastatic Breast Cancer Receiving CDK 4/6 Inhibitor Therapy

CDK 4/6 İnhibitörü ile Tedavi Edilen Metastatik Meme Kanseri Tanılı Hastalarda NOLUS Skorunun Prognostik Önemi

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ABSTRACT

Aim: Breast cancer is the most common cancer, and in the metastatic hormone-positive group, CDK 4/6 inhibitors are used. We aimed to examine the prognostic value of the "NOLUS" score, calculated from the estrogen receptor (ER), progesterone receptor (PR), and Ki-67 index, in patients with metastatic breast cancer receiving CDK 4/6 inhibitors.

Materials and Methods: This retrospective single-center study included 76 patients with HR+/HER2- metastatic breast cancer who were treated with CDK 4/6 inhibitors (ribociclib or palbociclib) and endocrine therapy between 2018 and 2024. The NOLUS score was calculated using the following formula: $-0.45 \times \text{ER\%} - 0.28 \times \text{PR\%} + 0.27 \times \text{Ki67\%} + 73$. A population-specific cut-off was determined via ROC analysis based on 2-year progression-free survival (PFS), classifying patients into "low-NOLUS" or "high-NOLUS" groups.

Results: The median patient age was 58.2 years. Ribociclib and palbociclib were used in 78.9% and 21.1% of the patients, respectively. During a median follow-up of 38.4 months, no significant difference was observed in the median PFS between the low- and high-NOLUS groups (36.6 vs. 27.9 months; $p=0.265$). Similarly, 48-month overall survival (OS) rates were comparable (50.4% vs. 59.1%; $p=0.772$). Multivariate analysis revealed that palbociclib use was independently associated with a higher risk of progression than ribociclib use (HR, 2.12; $p=0.026$). ECOG performance status and Ki-67 index were identified as independent prognostic factors for OS.

Conclusion: The NOLUS score was not a significant predictor of survival in patients with metastasis treated with CDK 4/6 inhibitors. However, this study provides real-world evidence regarding the differences in efficacy between CDK 4/6 inhibitors and highlights the prognostic value of ECOG status and Ki-67.

Keywords: Metastatic breast cancer, CDK 4/6 inhibitors, NOLUS score, Ribociclib, Palbociclib.

ÖZ

Amaç: Bu çalışmada, CDK 4/6 inhibitörü alan metastatik meme kanseri hastalarında; östrojen reseptörü (ER), progesteron reseptörü (PR) ve Ki-67 indeksinden hesaplanan "NOLUS" skorunun prognostik değerini incelemeyi amaçladık.

Gereç ve Yöntem: Bu retrospektif tek merkezli çalışmada, 2018-2024 yılları arasında CDK 4/6 inhibitörü (ribosiklib veya palbosiklib) ve endokrin tedavi kombinasyonu ile tedavi edilen 76 HR+/HER2- metastatik meme kanseri hastası dahil edildi. NOLUS skoru, $-0.45 \times \text{ER\%} - 0.28 \times \text{PR\%} + 0.27 \times \text{Ki67\%} + 73$ formülü kullanılarak hesaplandı. 2 yıllık progresyonsuz sağkalım (PSK) verileri baz alınarak yapılan ROC analizi ile popülasyona özgü bir kesim değeri (cut-off değeri) belirlendi ve hastalar "düşük-NOLUS" veya "yüksek-NOLUS" olarak sınıflandırıldı.

Bulgular: Hastaların medyan yaşı 58,2 yıldır. Ribosiklib ve palbosiklib kullanım oranları sırasıyla %78,9 ve %21,1 idi. 38,4 aylık medyan takip süresince, düşük ve yüksek NOLUS grupları arasında medyan PFS açısından istatistiksel olarak anlamlı bir fark gözlenmedi (36,6 aya karşı 27,9 ay; $p=0,265$). Benzer şekilde, 48 aylık genel sağkalım (OS) oranları da gruplar arasında benzer bulundu (%50,4'e karşı %59,1; $p=0,772$). Çok değişkenli analizde, palbosiklib kullanımının ribosiklibe kıyasla daha yüksek progresyon riski ile bağımsız olarak ilişkili olduğu saptandı (HR: 2.12, $p=0.026$). ECOG performans durumu ve Ki-67 indeksi, OS için bağımsız prognostik faktörler olarak belirlendi.

Sonuç: NOLUS skoru, CDK 4/6 inhibitörü ile tedavi edilen metastatik hastalarda sağkalımın anlamlı bir öngördürücüsü olarak saptanmamıştır. Bununla birlikte çalışma, CDK 4/6 inhibitörleri arasındaki etkinlik farklarına dair gerçek yaşam verileri sunmakta ve ECOG durumu ile Ki-67'nin prognostik değerini vurgulamaktadır.

Anahtar Kelimeler: Metastatik meme kanseri, CDK 4/6 inhibitörleri, NOLUS skoru, ribosiklib, palbosiklib.

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Introduction

Breast cancer is the most common malignancy among women worldwide and remains a leading cause of cancer-related mortality. It is a biologically heterogeneous disease, encompassing multiple subtypes with distinct molecular features, clinical behavior, and therapeutic responses.

Within this spectrum, Hormone Receptor (HR)-positive and Human Epidermal Growth Factor Receptor 2 (HER2)-negative subtypes constitute the most substantial patient cohort, accounting for approximately 70% of all breast cancer cases [1]. Historically, the predominant approach to managing this patient population has been endocrine therapy, including medications such as tamoxifen, aromatase inhibitors, and fulvestrant, which are designed to intervene with estrogen-dependent growth mechanisms of the tumor. Nevertheless, the development of primary or secondary resistance mechanisms to endocrine therapies has emerged as the most significant impediment to long-term disease control in patients with metastatic disease.

A more thorough understanding of the molecular mechanisms underlying cell cycle regulation, particularly the pivotal role of cyclin-dependent kinase (CDK) 4 and CDK 6 proteins in tumor proliferation, has led to a paradigm shift in treatment methodologies. CDK4 and CDK6 form a complex with cyclin D, leading to phosphorylation of the retinoblastoma (Rb) protein and progression from the G1 to S phase of the cell cycle. Dysregulation of this pathway is common in HR-positive breast cancer, providing the biological rationale for the use of CDK4/6 inhibitors. Hyperactivation of this pathway has been observed in a significant number of hormone receptor-positive breast cancer cases. CDK 4/6 inhibitors (e.g., palbociclib, ribociclib, and abemaciclib) have been developed to target this mechanism. When these inhibitors are combined with endocrine therapy, they inhibit the phosphorylation of Rb protein, thereby arresting the cell cycle in the G1 phase and driving tumor cells to senescence/apoptosis [2].

Phase III clinical trials conducted over the past decade (PALOMA, MONALEESA, MONARCH, and MONARCHE series) have demonstrated that the combination of CDK 4/6 inhibitors with aromatase

inhibitors or fulvestrant statistically and clinically significantly prolonged progression-free survival (PFS) compared to endocrine therapy alone [3–6]. Phase III trials (PALOMA, MONALEESA, and MONARCH and MONARCHE series) have consistently demonstrated that combining CDK4/6 inhibitors with endocrine therapy significantly prolongs progression-free survival compared with endocrine therapy alone [3–6]. Moreover, ribociclib and abemaciclib have shown a clear overall survival benefit, establishing CDK4/6 inhibitors as the standard of care in first- and second-line treatment of metastatic HR+/HER2– breast cancer [7].

Despite these successes, considerable challenges remain in clinical practice. Approximately 15–20% of patients demonstrate primary resistance to CDK 4/6 inhibitor therapy and experience disease progression within the initial 6 months. In most of the remaining patients, acquired resistance developed over time [8]. In light of the adverse effects associated with these medications, including neutropenia, hepatotoxicity, diarrhea, and QTc prolongation, in addition to the financial burden of treatment, it is imperative to accurately predict which patients will derive the greatest benefit from this therapeutic intervention and which patients will exhibit a more recalcitrant response, necessitating alternative treatments, such as chemotherapy. In the current state of clinical practice, the conventional biomarkers employed, namely the estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki-67, are inadequate for the accurate prediction of treatment response on an individual patient basis.

Determination of the intrinsic molecular subtypes of breast cancer (Luminal A, Luminal B, HER2-enriched, Basal-like) through gene expression profiling is considered the gold standard for elucidating the biology of the disease. A considerable proportion of tumors (10–20%) that are identified as HR+/HER2– through immunohistochemistry are known to possess “Non-Luminal” (HER2-enriched or Basal-like) characteristics at the genetic level. It has been established that this specific group exhibits resistance to endocrine therapy yet demonstrates heightened sensitivity to chemotherapy [9]. Nevertheless, the elevated expense and technical

intricacies inherent to genetic tests such as PAM50 constrain their conventional utilization.

To address this challenge, Pascual et al. [10] devised a mathematical model that employs three fundamental parameters (ER, PR, and Ki-67) that are already accessible in standard pathology reports: the Non-Luminal Disease Score (NOLUS). The objective of this score is to quantify the likelihood of a tumor having an aggressive “non-luminal” biology. This was achieved by combining low ER and PR expression levels with a high Ki-67 index. A high NOLUS score is theoretically characterized by low hormone receptor expression and a high proliferation rate. This score is associated with poor prognosis and endocrine resistance.

A review of the literature revealed variable results in studies investigating the prognostic value of the NOLUS score. While the NOLUS score has been demonstrated to successfully predict the response to neoadjuvant chemotherapy (pathological complete response, pCR) in early stage breast cancer, its ability to predict survival in the metastatic setting, particularly in the presence of CDK 4/6 inhibitors, remains unclear. The association between a high score and poor PFS and OS has been reported in some studies [11]; however, others (e.g., everolimus studies) have failed to demonstrate this association [12]. Furthermore, there is no consensus on the cut-off values used in calculating the score; the threshold value of 51.38 recommended in the original study may not be ideal for every population.

Study Objective and Hypothesis

Although CDK 4/6 inhibitors are widely used in Türkiye, data on cost-effective biomarkers to predict their efficacy in the Turkish population remain limited. We hypothesized that the NOLUS score, calculated from standard immunohistochemical (IHC) parameters, could serve as a prognostic indicator for patients with metastatic HR+/HER2-breast cancer receiving CDK 4/6 inhibitors. Therefore, the primary objective of this study was to evaluate the impact of the NOLUS score on progression-free survival (PFS) and overall survival (OS) in our real-world clinical cohort. Additionally, we aimed to determine whether a population-specific cut-off value differs from those

reported in the literature and how this might affect clinical outcomes. Finally, we assessed whether the specific CDK 4/6 inhibitor agent (palbociclib versus ribociclib) and other clinicopathological factors, such as Eastern Cooperative Oncology Group (ECOG) performance status and sites of metastasis, independently influenced survival outcomes.

Materials and Methods

Study Population

We retrospectively evaluated patients diagnosed with hormone receptor (HR)-positive and human epidermal growth factor receptor 2 (HER2)-negative metastatic breast cancer who received cyclin-dependent kinase 4/6 (CDK 4/6) inhibitor therapy. The study cohort consisted of patients who were routinely followed at the Medical Oncology Clinic of Antalya Training and Research Hospital between February 2018 and August 2024.

The inclusion criteria for the study were as follows: patients who were 18 years of age or older, had a confirmed diagnosis of metastatic breast cancer, and were receiving CDK 4/6 inhibitor therapy. The exclusion criteria were a lack of access to patient follow-up data, a diagnosis of HER2-positive breast cancer, or a diagnosis of HR-negative breast cancer. Seventy-six patients who met the inclusion criteria were included in the final analysis (n=76). All patients were treated using the most appropriate methods and agents according to current treatment guidelines.

An application was submitted to the Local Scientific Research Ethics Committee to conduct the study, and the necessary ethical approval was obtained on 23.10.2025 (decision number 18/9).

Study Design and Data Collection

The study encompassed a range of demographic and clinical data, including patient age, sex, menopausal status, Eastern Cooperative Oncology Group Performance Status (ECOG PS), treatments administered for breast cancer diagnosis, and treatment responses. Pathological examination was conducted, focusing on the evaluation of ER, PR, and HER2 status, along with the Ki-67 index. The findings were documented by referencing the pathology reports

that substantiated the diagnosis of breast cancer. In accordance with the established criteria, HER2 status was deemed positive in cases where a score of 2+ was accompanied by positive in situ hybridization results by IHC, or where a score of 3+ was ascertained through IHC. Patients with these characteristics were excluded from this study.

The percentage values of the ER, PR, and Ki-67 indices by IHC were recorded and used for NOLUS calculations. NOLUS was determined using the following formula: $NOLUS = -0.45 \times ER(\%) - 0.28 \times PR(\%) + 0.27 \times Ki67(\%) + 73$, which was derived from the published literature [10]. This study aimed to determine the ideal cut-off value for NOLUS. To accomplish this, the Receiver Operating Characteristic (ROC) curve based on the 2-year landmark PFS rate of the study cohort was used. Patients were dichotomized into low- and high-NOLUS groups according to the determined cut-off value.

Breast cancer staging was accomplished using the American Joint Committee on Cancer (AJCC) Staging System, 8th edition.

A comprehensive evaluation of patient responses to CDK 4/6 inhibitor therapy was conducted through a meticulous review of imaging data acquired using the Response Evaluation Criteria in Solid Tumors (RECIST, v1.1) criteria. Patients who demonstrated at least a partial response to treatment were categorized as having an objective response, whereas those who exhibited stable disease (SD) were considered to have achieved disease control.

PFS was defined as the duration between the commencement of CDK 4/6 inhibitor therapy and the onset of progression, or, for participants who did not exhibit progression, the most recent follow-up. OS was defined as the interval between the initiation of CDK 4/6 inhibitor treatment and death or the most recent follow-up.

Statistical Analysis

The data obtained in this study were analyzed using IBM SPSS V.24.0. Continuous variables are expressed as median values (min-max) in parentheses, while categorical variables are

expressed as percentage frequencies. The Chi-square test was used to compare categorical variables between the low- and high-NOLUS groups. The Cox proportional hazards regression method was employed in both univariate and multivariate analyses to ascertain the impact of demographic, clinical, and pathological factors on survival. Survival curves were delineated using the Kaplan-Meier method. Variables exhibiting a p-value <0.3 in the univariate analyses were incorporated into the multivariate analysis. The hazard ratio (HR) was computed for each variable with a 95% confidence interval (CI). A p-value of less than 0.05 was established as the threshold for statistical significance.

Results

The final analyses were performed on 76 patients diagnosed with metastatic breast cancer (n = 76). The median age of the patients was 58.2 (31.5-85.1), and all patients were female.

Upon examination by CDK4/6 type, 60 patients (78.9%) received ribociclib, while 16 patients (21.1%) received palbociclib. None of the patients had received abemaciclib. As indicated by the 24.47 cut-off NOLUS value determined through ROC analysis, 31 patients (40.8%) exhibited low NOLUS values, and 45 patients (59.2%) demonstrated high NOLUS values. Upon dichotomizing the patients based on their NOLUS values, the clinical and pathological characteristics were evenly distributed between the two groups.

(See Table 1) The median follow-up duration for patients was 38.4 months.

During the follow-up period, 44 patients (57.9%) exhibited breast cancer progression and 25 (32.9%) succumbed to the disease. Objective response rates (ORR) and disease control rates (DCR) were numerically higher in patients with low NOLUS values; however, these differences were not statistically significant (Figure 1).

Patients with low and high NOLUS scores demonstrated comparable median progression-free survival (PFS) durations of 36.6 months (95% confidence interval [CI]: 32.0-41.3) and 27.9 months (95% CI: 20.9-34.9), respectively. (log-rank test, p=0.265). Although the median

overall survival (OS) values were not attained in the low and high NOLUS value patient groups, the 48-month OS was 50.4% in patients with low NOLUS values and 59.1% in patients with high NOLUS values (log-rank, $p=0.772$) (Figure 2).

In the course of examining the variables that affected PFS, the NOLUS score was determined to be neither a significant nor an independent prognostic factor. Nevertheless, the administration of palbociclib, a CDK 4/6 inhibitor, was associated with an elevated risk of progression compared with ribociclib [HR 2.12 (95% CI, 1.10-4.09); $p=0.026$] (Table 2). Similarly, the NOLUS score did not emerge as an independent prognostic variable for overall survival (OS). However, the Eastern Cooperative Oncology Group performance status (ECOG PS) and Ki-67 index were identified as variables that exhibited independent prognostic significance for OS (Table 3).

Table 1. Clinicopathological Characteristics of the Patients According to NOLUS

	Low NOLUS (n=31) n (%)	High NOLUS (n=45) n (%)	P value
Age (years)			0.253
≤50	6 (19.4)	14 (31.1)	
>50	25 (80.6)	31 (68.9)	
ECOG PS			0.337
0-1	26 (83.9)	41 (91.1)	
2	5 (16.1)	4 (8.9)	
Menopausal Status			0.067
Premenopausal	4 (12.9)	14 (31.1)	
Peri/Postmenopausal	27 (87.1)	31 (68.9)	
Estrogen Receptor			0.142
≤60	0 (0.0)	3 (6.7)	
>60	31 (100)	42 (93.3)	
Ki-67 Index (%)			0.052
≤20	15 (48.4)	12 (26.7)	
>20	16 (51.6)	33 (73.3)	
CDK 4/6 Inhibitor Agent			0.399
Ribociclib	23 (74.2)	37 (82.2)	
Palbociclib	8 (25.8)	8 (17.8)	
Hormonal Therapy			0.97
Tamoxifen/AI	24 (77.4)	35 (77.8)	
Fulvestrant	7 (22.6)	10 (22.2)	

ECOG PS: Eastern Cooperative Oncology Study Group Performance Status

Table 2. Univariate and Multivariate Analyses for Progression-Free Survival

	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Age (years)		0.068		
≤50	Reference			
>50	0.52 (0.26-1.06)			
ECOG PS		0.314		
0-1	Reference			
2	1.52 (0.67-3.43)			
Menopausal Status		0.445		
Premenopausal	Reference			
Peri/Postmenopausal	0.74 (0.35-1.59)			
Ki-67 Index (%)		0.095		0.051
≤20	Reference		Reference	
>20	0.60 (0.34-1.10)		0.54 (0.29-1.00)	
NOLUS		0.265		0.073
Low	Reference		Reference	
High	1.42 (0.76-2.65)		1.83 (0.95-3.55)	
CDK 4/6 Inhibitor Agent		0.069		0.026
Ribociclib	Reference		Reference	
Palbociclib	1.79 (0.96-3.34)		2.12 (1.10-4.09)	
Hormonal Therapy		0.830		
Tamoxifen/AI	Reference			
Fulvestrant	1.08 (0.54-2.16)			

CI: Confidence Interval, ECOG PS: Eastern Cooperative Oncology Study Group Performance Status, HR: Hazard Ratio

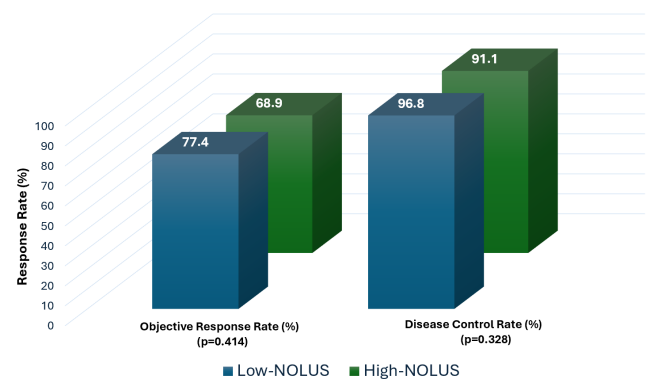


Figure 1: CDK 4/6 Inhibitor response rates according to NOLUS levels

Table 3. Univariate and Multivariate Analyses for Overall Survival

		Univariate Analysis		Multivariate Analysis	
		HR (95% CI)	P value	HR (95% CI)	P value
Age (years)			0.809		
≤50	Reference				
>50	0.89 (0.35-2.28)				
ECOG PS			0.033		0.049
0-1	Reference			Reference	
2	2.78 (1.09-7.13)			2.61 (1.01-6.80)	
Menopausal Status			0.828		
Premenopausal	Reference				
Peri/Postmenopausal	0.90 (0.33-2.43)				
Ki-67 Index (%)			0.011		0.015
≤20	Reference			Reference	
>20	0.35 (0.16-0.79)			0.37 (0.16-0.82)	
NOLUS			0.772		0.231
Low	Reference			Reference	
High	1.12 (0.51-2.48)			1.70 (0.71-4.06)	
CDK 4/6 Inhibitor Agent			0.601		
Ribociclib	Reference				
Palbociclib	1.25 (0.54-2.93)				
Hormonal Therapy			0.528		
Tamoxifen/AI	Reference				
Fulvestrant	1.33 (0.55-3.21)				

CI: Confidence Interval, ECOG PS: Eastern Cooperative Oncology Study Group Performance Status, HR: Hazard Ratio

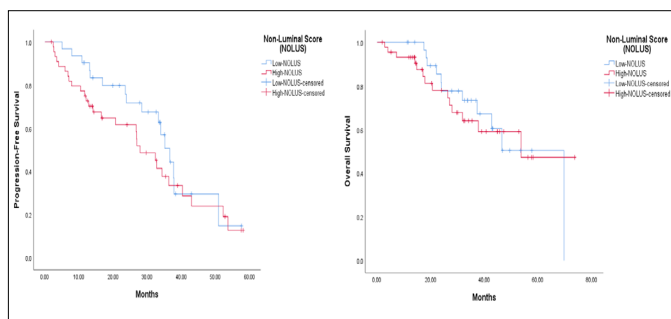


Figure 2: Progression-free survival and overall survival according to NOLUS levels

Discussion

Although the NOLUS score was developed as a

practical and accessible surrogate for identifying aggressive, endocrine-resistant 'non-luminal' disease when genomic testing is unavailable [10], its real-world prognostic value in the metastatic setting requires further clinical validation. In this context, our findings indicate that the NOLUS score was not a statistically significant prognostic factor for progression-free survival (PFS) or overall survival (OS) in a cohort of 76 Turkish patients with metastatic disease receiving CDK 4/6 inhibitors.

Our findings indicate that the NOLUS score is not a statistically significant prognostic factor for progression-free survival (PFS) or overall survival (OS) in a cohort of 76 Turkish patients with metastatic disease receiving CDK 4/6 inhibitors. The median progression-free survival (PFS) duration was 36.6 months in patients with a low NOLUS score who were expected to have a better prognosis. In contrast, the median PFS duration was 27.9 months for patients with a high NOLUS score. Despite the approximately 9-month discrepancy being clinically significant, the absence of statistical significance ($P = 0.265$) can be attributed to multiple factors.

The inclusion of 76 patients in the study might have been inadequate, particularly for subgroup analyses and statistical demonstration of survival differences. Negative results in similar studies within the existing body of literature have also generally been reported in small to medium-sized patient groups [13, 14].

The NOLUS score was originally developed based on data from patients with early stage breast cancer. Metastatic disease is characterized by a high degree of complexity, with key dynamics including tumor burden, metastatic sites, and prior treatment. The NOLUS score, derived from IHC data obtained from the primary tumor, may not fully reflect the biological evolution of metastatic processes.

Resistance to CDK 4/6 inhibitors is not solely associated with the "Non-luminal" phenotype. A growing body of research has identified several molecular mechanisms that contribute to drug resistance, including ESR1 mutations, PIK3CA mutations, cyclin E1, and Rb loss [15]. Given the limitations of the NOLUS score, which does

not account for these genetic factors, it may be insufficient to predict resistance accurately.

Consistent with the findings reported in the extant literature, Prat et al. [12] determined that the NOLUS score was not significantly associated with either progression-free survival (PFS) or overall survival (OS) in their study of patients with metastatic disease receiving everolimus treatment. Nevertheless, in early stage neoadjuvant chemotherapy studies (Amylidi et al.) [16], a high NOLUS score was associated with better pCR. This suggests that the NOLUS score is successful in reflecting the proliferation rate, but has a limited ability to predict the efficacy of CDK 4/6 inhibitors in the metastatic setting.

A particularly noteworthy methodological aspect of the present study was the cut-off value employed. In the original study by Pascual et al. [10] and numerous subsequent publications, the cut-off value was established at 51.38. Patients exhibiting values exceeding this threshold were classified as “high-risk/non-luminal.”

However, the use of a universally applicable cut-off value in biomarker studies may lead to inaccurate outcomes. This is due to various factors, including disparities in immunohistochemical (IHC) staining methodologies employed by different laboratories, the subjective nature of pathologist evaluations, and the influence of population genetics, all of which can impact the distribution of scores. The value of 24.47 ascertained through receiver operating characteristic (ROC) analysis in our study, exhibited a substantial discrepancy compared to the initial value.

This may indicate that patients in our study population generally exhibited lower Ki-67 values or higher ER/PR values, resulting in a lower overall average score. The failure to achieve statistical significance despite the use of a low threshold value, such as 24.47, confirmed that the discriminatory power of the score was weak within this particular population. In our study, a population-specific cut-off value of 24.47 was established using ROC analysis to optimally evaluate the cohort. This value is notably lower than the 51.38 threshold originally proposed by Pascual et al. [10]. It is crucial to emphasize that the original NOLUS score was derived from patients

with early-stage breast cancer. The biological dynamics, prior treatment exposures, and tumor evolution in metastatic disease are fundamentally different. When we applied the original 51.38 cut-off to our cohort as an additional analysis, a highly skewed distribution was observed, with the vast majority of patients falling into the 'Low-NOLUS' category. This significant discrepancy suggests that cut-offs derived from early-stage disease may not be directly extrapolatable to the metastatic setting, highlighting the necessity of stage-specific thresholds in clinical practice. Moreover, conducting a statistical analysis would have been unfeasible. This illustrates that the clinical applicability of the NOLUS score is constrained by challenges related to standardization.

In our multivariate analysis, palbociclib use was associated with an increased risk of progression compared to ribociclib (HR: 2.12, $p=0.026$). However, the patient distribution between the two treatment arms was highly uneven (78.9% ribociclib vs. 21.1% palbociclib). Because of this imbalance and the retrospective study design, this survival difference should be interpreted cautiously. Patient selection bias may have influenced physician preferences and, consequently, these clinical outcomes. This study, rooted in a single-center dataset, provides a significant contribution to the ongoing dialogue surrounding the clinical implications of these inhibitors.

A synthesis of clinical trial data revealed that ribociclib is the sole agent that consistently demonstrated advantages in both progression-free survival (PFS) and overall survival (OS) across the entirety of the MONALEESA-2, 3, and 7 trials [7, 17]. In contrast, palbociclib exhibited a PFS advantage in the PALOMA-2 trial but failed to attain statistical significance in OS data [18].

A review of the existing literature reveals mixed results. Large US database studies, such as those conducted by Flatiron Health, suggest the absence of statistical differences between agents [19]. Conversely, several studies have reported survival differences in favor of ribociclib, consistent with the findings of the present study [7, 20].

Recent real-world studies have evaluated the outcomes of CDK 4/6 inhibitors in Turkish

patients. Yildirim et al. [21] reported the efficacy and manageable toxicity of first-line CDK 4/6 inhibitors in premenopausal patients. Demir et al. [22] observed similar clinical benefits with palbociclib in heavily pretreated cases. Regarding post-progression treatments, Beypinar et al. [23] evaluated the efficacy of everolimus combined with endocrine therapy after CDK 4/6 inhibitor failure. While these studies provide valuable data on treatment efficacy and patient management in our population, our research specifically focused on evaluating the NOLUS score as a prognostic tool.

There are several potential explanations for the discrepancy between our study results and those of previous studies. These include the pharmacological properties of the drugs (owing to the higher selectivity of ribociclib for CDK4), patient compliance, and patient selection bias in the study group. For instance, physicians may have opted to prescribe palbociclib to patients with a poorer prognosis or elevated comorbidities attributable to the perceived side effect profile. Nevertheless, the persistence of the discrepancy, even after adjustment for performance status (ECOG) and additional factors in the multivariate analysis, suggests a potential molecular-level divergence in efficacy.

In the present study, ECOG PS was identified as a significant prognostic factor for OS, consistent with the established oncological principles (HR: 2.61). This result substantiates the fundamental tenet in oncology that a patient's physiological reserve serves as the primary determinant of treatment efficacy. However, findings concerning the Ki-67 index may initially seem contradictory. Elevated Ki-67 levels (>20%) have been shown to be indicative of a more favorable prognosis for overall survival (OS) (HR: 0.37, $p=0.015$). In general, high proliferation is indicative of poor prognosis. One possible explanation for this result is that tumors with high Ki-67 (Luminal B-like) levels demonstrate a greater response to cytotoxic chemotherapy (taxanes and anthracyclines) when administered subsequent to CDK 4/6 inhibitor therapy. While tumors exhibiting low Ki-67 (Luminal A) may demonstrate resistance to chemotherapy, the sensitivity to chemotherapy exhibited by tumors with high proliferation may

have contributed positively to overall survival in the long term. Because of the retrospective design and limited sample size, our finding regarding the high Ki-67 index and overall survival is primarily hypothesis-generating. Larger prospective trials are needed to confirm whether high proliferation rates predict a better response to subsequent chemotherapy.

Limitations of the Study

Several limitations of this study should be considered when evaluating its results. First, the retrospective study design carries the risk of data loss and selection bias. Second, the single-center nature of the study and the sample size of 76 patients limited its generalizability. Furthermore, IHC staining may have been evaluated by different pathologists and no central review was performed on the data.

There are additional limitations to consider. First, our cohort did not include any patients treated with abemaciclib, which prevents a comparison across all three CDK 4/6 inhibitors. Second, we did not analyze the prognostic effect of different metastatic sites. Finally, the variation in subsequent treatments after disease progression was not evaluated, and this difference might have affected the overall survival outcomes.

Finally, calculating a population-specific cut-off value via ROC analysis in a small sample size restricts the generalizability of our results. The threshold of 24.47 requires validation in larger, independent cohorts before any clinical application.

Conclusion

In summary, the findings of this single-center retrospective study indicate that the NOLUS score is not an independent prognostic marker for either progression-free survival (PFS) or overall survival (OS) in patients with metastatic human epidermal growth factor receptor 2 negative (HER2-negative) breast cancer receiving cyclin-dependent kinase 4/6 (CDK4/6) inhibitor therapy.

Although the numerical differences observed between the NOLUS score groups suggest a potential biological basis for the score, the current formula and cut-off values do not provide sufficient

evidence for routine use in clinical practice.

The most salient secondary finding of our study was real-world data illuminating the divergence in efficacy between Ribociclib and Palbociclib. The substantial PFS advantage observed in favor of ribociclib lends credence to the notion that drug-specific data should be given serious consideration when selecting therapeutic interventions. Furthermore, ECOG performance status and the Ki-67 index have been shown to be the most reliable factors for determining prognosis in this patient group. Future studies should focus on the development and validation of novel scoring systems in larger patient cohorts combined with standardized pathological assessments and genetic mutation analysis.

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