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Prognostic Value of Cyclin D1 Overexpression in Invasive Breast Carcinoma

İnvaziv Meme Karsinomunda Cyclin D1 Aşırı Ekspresyonunun Prognostik Değeri

Erdem ÇOMUT[1]
Demet KOCATEPE ÇAVDAR[2]
Ayşe YAĞCI[3]
Enver VARDAR[4]
Funda TAŞLI[2]

[1]Department of Pathology, Pamukkale University, Faculty of Medicine, Denizli, Türkiye
[2]Department of Pathology, İzmir City Hospital, İzmir, Türkiye
[3]Frozen Pathology Laboratory, İzmir, Türkiye
[4]Bozyaka Educational and Research Hospital, İzmir, Türkiye

ABSTRACT

Objective: Breast cancer is the most diagnosed cancer worldwide, accounting for 11.7% of all cancers. Cyclin D1, a regulator of CDK4/6 and cell cycle progression, functions as an oncogene through overexpression, contributing to the pathogenesis of cancers including breast carcinoma. This study aimed to evaluate Cyclin D1 expression and its clinicopathological significance in invasive breast carcinoma within the Turkish population.

Methods: H&E-stained and immunohistochemical preparations from partial and total mastectomy specimens of 143 patients, diagnosed between 2007 and 2013, were examined. Cyclin D1 overexpression was evaluated in 1000 cells using an IHC score based on nuclear staining intensity (0-3) and the percentage of positive tumor cells (1-3), classified as weak ('+' or '++') or strong positive ('+++'). Pearson's Chi-Square and Spearman's rho were used to analyze the relationship between Cyclin D1 expression and clinicopathological parameters statistically.

Results: The median age of the patients was 58.5 years (Min:28, Max:92). Cyclin D1 status showed no correlation with age, T stage, or lymph node metastasis. However, a moderate positive correlation was observed with ER (r = 0.32, *P* <.001) and PR (r = 0.31, *P* <.001) scores. Among molecular subgroups, Cyclin D1 overexpression was most significant in Luminal B group (92.9%, *P* = .008), while Triple-negative group showed significantly lower overexpression (40%, *P* = .008).

Conclusion: Cyclin D1 overexpression in Luminal B and Luminal A groups, along with its positive correlation with ER, suggests its role in estrogen-sensitive breast cancer pathogenesis. Strong Cyclin D1 overexpression was associated with reduced survival time in HER2-positive cases.

Keywords: Breast, invasive breast carcinoma, Cyclin D1, histopathology, immunohistochemistry

ÖZ

Amaç: Meme kanseri, dünya genelinde en sık tanı konulan kanser olup tüm kanserlerin %11,7'sini oluşturmaktadır. Cyclin D1, CDK4/6 ve hücre döngüsü ilerlemesini düzenleyen bir onkogen olarak aşırı ekspresyon yoluyla meme karsinomu da dahil olmak üzere çeşitli kanserlerin patogenezine katkıda bulunmaktadır. Bu çalışmada, Türk popülasyonunda invaziv meme karsinomunda Cyclin D1 ekspresyonu ve klinikopatolojik önemi değerlendirildi.

Yöntemler: 2007-2013 yılları arasında tanı konulan 143 hastaya ait parsiyel ve total mastektomi örneklerinden hazırlanan H&E ve immünohistokimyasal preparatlar incelendi. Cyclin D1 aşırı ekspresyonu, nükleer boyanma şiddetine (0-3) ve pozitif tümör hücrelerinin yüzdesine (1-3) dayalı IHC skoru kullanılarak 1000 hücrede değerlendirildi. Bulgular zayıf pozitif ('+' veya '++') veya güçlü pozitif ('+++') olarak sınıflandırıldı. Cyclin D1 ekspresyonu ile klinikopatolojik parametreler arasındaki ilişki, Pearson Ki-Kare ve Spearman rho testi kullanılarak analiz edildi.

Bulgular: Hastaların medyan yaşı 58,5 yıl (Min: 28, Maks: 92) idi. Cyclin D1 durumu ile yaş, T evresi veya lenf nodu metastazı arasında anlamlı bir ilişki bulunmadı. Ancak, ER (r = 0,32, *P* < ,001) ve PR (r = 0,31, *P* <, 001) skorlarıyla orta derecede pozitif bir korelasyon gözlendi. Moleküler alt gruplar içinde, Cyclin D1 aşırı ekspresyonu en çok Luminal B

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Sorumlu Yazar/Corresponding author: Erdem ÇOMUT
E-mail: comuterdem@gmail.com
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grubunda (%92,9, *P* = ,008) saptandı ve Triple negatif grupta anlamlı derecede düşük ekspresyon (%40, *P* = ,008) gözlendi.
Sonuç: Cyclin D1'in Luminal A ve Luminal B gruplarında aşırı ekspresyonu ve ER ile pozitif korelasyonu, bu proteinin östrojen duyarlı meme kanseri patogenezindeki rolünü düşündürmektedir. HER2-pozitif olgularda güçlü Cyclin D1 aşırı ekspresyonu, azalmış sağkalım süresi ile ilişkili bulunmuştur.
Anahtar Kelimeler: Meme, invaziv meme karsinomu, Cyclin D1, histopatoloji, immünohistokimya

INTRODUCTION

Breast cancer stands as the most frequently diagnosed cancer globally, with 2.3 million new cases reported in 2020, representing 11.7% of all cancer diagnoses. It ranks as the fifth most prevalent cause of cancer-related mortality, accounting for 6.9%, and exhibits higher mortality rates in developing countries when contrasted with developed nations.[1] Like global trends, breast cancer is the most prevalent cancer among women also in Turkey, with around 12,000 new cases diagnosed in 2018.[2] The breast cancer is associated with well-established risk factors such as age, family history, early menarche, late menopause, nulliparity, late age at first pregnancy, and hormone replacement therapy.

Histopathologically, invasive breast carcinoma (IBC) displays notable heterogeneity and is categorized into various subtypes, including invasive carcinoma of no special type, invasive lobular carcinoma (ILC), and others. The assessment of hormone receptor status, involving estrogen receptor (ER) and progesterone receptor (PR), through immunohistochemical methods is pivotal for identifying patients eligible for endocrine therapy. HER2 immunohistochemistry (IHC) and in-situ hybridization evaluation are also essential for identifying patients eligible to receive anti-HER2 therapy.[3] Per current protocols, essential elements for the pathological reporting of resected materials include tumor size, histological type, histological grade, lymphovascular invasion (LVI), tumor-infiltrating lymphocytes (TILs), multifocality, lymph node status, presence of in-situ components, and surgical margins. IBC is molecularly classified into four groups: luminal A, luminal B, HER2-enriched, and Basal-like triple-negative (triple-negative breast cancer, TNBC). The integration of morphologic features and molecular profiling is intended to enhance the clinical outcomes of patients.[4]

The cell cycle is meticulously controlled by cyclins and cyclin-dependent kinases (CDKs). Cyclin D1, which functions as a mitogenic sensor and activator of CDK4/6, plays a pivotal role in the progression of the cell cycle. The overexpression, accumulation, or improper cellular localization of the Cyclin D1 protein results in its acting as an oncogene.[5] Cyclin D1 has been implicated in the pathogenesis of various neoplasms, including breast carcinoma. It is involved in both the normal lobuloalveolar development of the breast and the process of breast carcinogenesis.[6] Recently, first-line therapy for postmenopausal patients with HR-positive, HER2-negative recurrent/stage IV breast cancer includes the use of CDK 4/6 inhibitors in combination with aromatase inhibitors. Similarly, for premenopausal patients undergoing ovarian ablation/suppression, this therapeutic combination is recommended.[7]

In our study, our objective was to retrospectively assess the clinical and histopathologic data of patients with IBC and elucidate the potential impact of Cyclin D1 overexpression on patient prognosis.

METHODS

Ethical approval for the study was obtained from the İzmir Bozyaka Education and Research Hospital Ethics Committee on March 25, 2021 (Meeting No. 2, Decision No. 121). From 2007 to 2013, a total of 158 cases diagnosed with breast cancer in partial or total mastectomy specimens were initially identified. Six cases, where paraffin blocks were inaccessible, and nine cases lacking sufficient tumor tissue due to technical issues during tissue microarray (TMA) block preparation, were excluded. Consequently, a total of 143 cases were included in the study. All cases were specifically diagnosed with IBC, with the exclusion of rare and salivary gland-type tumors, neuroendocrine neoplasms, mesenchymal tumors of the breast, fibroepithelial tumors, nipple tumors, malignant lymphomas, or metastatic tumors. The clinical data were sourced from the electronic patient database of the hospital.

TMA Construction

Target regions on the tumor slides were labelled, and 4 mm diameter tumor samples were extracted from the paraffin blocks using a punch biopsy tool. These samples were then embedded in TMA blocks and prepared for sectioning. Immunohistochemical staining for Cyclin D1 (Clone GM, 1:50 dilution, Leica) was performed on 5-µm-

Table 1. Overview of immunohistochemical stains

Antibody	Clone	Type of antibody	Source	Dilution
Cyclin D1	GM	Mouse monoclonal	Leica	1/50
ER	6F11	Mouse monoclonal	Leica	1/50
PR	312	Mouse monoclonal	Leica	1/100
HER2	356	Mouse monoclonal	Leica	1/40-1/80
Ki-67	SP6	Rabbit monoclonal	TFS	1/100-1/200
p53	DO7	Mouse monoclonal	Leica	1/800

Abbreviations: ER: Estrogen receptor, HER2: Human epidermal growth factor receptor 2, PR: Progesterone receptor, TFS: Thermo fischer scientific.

thick sections taken on positively charged slides, using an automated immunohistochemical stainer according to the manufacturer's guidelines (streptavidin-peroxidase protocol, BenchMark; Ventana, PA).

Immunohistochemical Analysis

IHC slides, encompassing ER, PR, HER2, Ki-67, and p53, along with hematoxylin and eosin (H&E) stained slides, were retrieved from the pathology archive. Subsequently, they underwent light microscopic reevaluation by two pathologists, with expertise spanning more than 15 years and 1-5 years, respectively (FA and EC). Cyclin D1 overexpression observed as brown granules within the cell nucleus. A total of 1000 cells were assessed, and both staining intensity and the percentage of invasive tumor cells were analyzed. IHC score, derived from multiplying the staining intensity (0-none, 1-mild, 2-moderate, 3-intense) by the percentage (1- <10%, 2- 10-50%, 3- >50%), was used for classification: 0 points as '-'; 1-2 points as '+'; 3-4 points as '++'; and >4 points as '+++'. Overall, '+' and '++' are classified as weak positive, and '+++' as strong positive group.[8] ER and PR positivity was defined as ≥1% of tumor cell nuclei showing specific staining for the receptor. Cases with 1–10% of nuclear staining for ER were classified as "low positive," while those with >10% were considered "positive." Negative cases were defined as <1% of tumor cell nuclei staining for the receptor. Both intensity (weak, moderate, or strong) and the percentage of positive cells were recorded.[9] HER2 positivity was defined as strong, complete membrane staining in >10% of tumor cells (IHC score 3+). An equivocal result (IHC score 2+) was characterized by weak to moderate, complete membrane staining in >10% of tumor cells or strong, complete staining in ≤10% of tumor cells. Cases with incomplete, faint/barely perceptible membrane staining in >10% of tumor cells (IHC score 1+) or no staining at all (IHC score 0) were considered HER2-negative.[10] HER2 status was assessed solely using immunohistochemical (IHC) analysis. FISH (Fluorescence In Situ Hybridization) was not performed in this study. Ki-67 and p53 staining in tumor cells were noted as percentages. The immunohistochemical markers were detailed in Table 1. Luminal A breast cancers were defined by the expression of estrogen receptors (ER) and/or progesterone receptors (PR), along with a low Ki-67 index (typically less than 14%) and the absence of HER2 overexpression. Luminal B breast cancers were characterized by positive estrogen receptor (ER) status, but with either a higher Ki-67 index (greater than 14%) or overexpression of HER2.

Statistical Analysis

IBM SPSS Statistics version 15 (IBM SPSS Corp., Armonk, NY, USA) was used for statistical analysis. Pearson's Chi-square test was conducted to compare Cyclin D1 expression with various clinical indicators and histopathologic parameters. Spearman's correlation method was employed to analyze the correlation between Cyclin D1 expression and various clinical parameters, including age at diagnosis, tumor size, clinical stage, and the status of ER, PR, and HER2. In this study, FISH analysis for confirmation could not be performed on cases with a HER2 score of 2+, and these cases were included in the HER2-positive group for statistical analysis. Overall survival (OS) and disease-free survival (DFS) were assessed using Kaplan-Meier analysis.

This study was conducted in accordance with the regulations outlined by the Helsinki Declaration. The study protocol was approved by the Ethics Committee of Izmir Bozyaka Educational and Research Hospital (Decision No: 4, Year: 2016).

RESULTS

Overall Patient Characteristics

The patients' ages ranged from 28 to 92 years, with a median age of 58.5. Most of the patients (n=142) were female, while only one (0.7%) was male. Axillary lymph node metastasis was observed in 86 cases (60.1%). The clinical features of the cases are detailed in Table 2. No statistically significant correlation was evident between Cyclin D1

Table 2. Clinical features of IBC cases

Feature	n	%
Sex (n=143)		
Female	142	99.3
Male	1	0.7
Age (n=143)		
<40	13	9.1
40-60	61	42.7
>60	69	48.2
Menopausal status (n=139)		
Premenopausal	38	26.6
Postmenopausal	100	69.9
Male	1	0.7
Tumor size (n=143)		
<2 cm	28	19.5
2-5 cm	93	65.1
>5 cm	22	15.4
Multiple foci (n=143)		
Present	24	16.8
Absent	119	83.2
T stage (n=143)		
1A	1	0.7
1B	4	2.8
1C	32	22.4
2	83	58.0
3	20	14.0
4A	2	1.4
4B	1	0.7
N stage (n=143)		
0	47	32.9
1A	36	25.2
1mi	9	6.3
2A	26	18.2
3A	25	17.5
Axillary lymph node metastasis (n=143)		
Present	86	60.1
Absent	57	39.9
Stage (TNM) (n=143)		
1	18	12.6
2A	31	21.7
2B	35	24.5
3A	20	14.0
3B	5	3.5
3C	22	15.4
4	8	5.6
Recurrence (n=135)		
Present	34	25.2
Absent	101	74.8
Chemotherapy (n=137)		
Present	105	76.6
Absent	32	23.4
Radiotherapy (n=141)		
Present	98	69.5
Absent	43	30.5

Abbreviations: pN1mi: Micrometastases consisting of approximately 200 cells, measuring larger than 0.2 mm but not exceeding 2.0 mm in size.

expression and key clinical parameters, namely patient age, menopausal status, tumor multifocality, lymph node metastasis, clinical stage, or treatment status, as detailed in Table 3 ($P > .05$).

Histopathological Features

Histopathologically, 88% of the cases (n=24) were classified as invasive breast carcinoma (IBC) of no special type (NST), followed by invasive lobular carcinoma (ILC) in 8 cases (6%), invasive micropapillary carcinoma in 5 cases (2%), metaplastic carcinoma in 3 cases (2%), tubular carcinoma in 2 cases (1%), and invasive papillary carcinoma in 1 case (1%). Most tumors were histologically graded as Grade 2 (n=81, 56.6%), with 16 cases (11.2%) being Grade 1 and 46 cases (32.2%) Grade 3. An in-situ component, including ductal carcinoma in situ (DCIS) or lobular carcinoma in situ (LCIS), was observed in 97 cases (68%). Tumor-infiltrating lymphocytes (TILs) were identified in 76 cases, with 28.9% (n=22) located in the stromal region and the majority (71.1%) as intratumoral TILs. LVI was detected in 92 cases (64.3%). Extranodal extension was observed in 50 (58.1%) of the 86 patients with axillary lymph node metastasis. No significant relationship was identified between Cyclin D1 and histological parameters, and the findings are summarized in Table 4.

Immunohistochemical Features

Cyclin D1 overexpression was observed as weakly positive in 81 cases (56.6%) and strongly positive in 38 cases (26.6%), while no expression was noted in 24 cases (16.8%). Figure 1-4 displays H&E and immunohistochemistry images illustrating cases with varying Cyclin D1 expression scores. Cyclin D1 showed a moderate positive correlation with ER ($r = 0.33$, $P < .001$) and PR ($r = 0.31$, $P < .001$), while no significant correlations were found with HER2 ($r = -0.058$) or p53 ($P = .371$) (Table 5). As detailed in Table 6, Cyclin D1 overexpression was observed in 92.9% of Luminal B cases and only 60% of Triple-negative cases ($P = .008$).

Survival Analysis

The cases were systematically followed up at 6-month

Table 3. The relationship between Cyclin D1 expression and clinical parameters of IBC cases (n=143)

	Cyclin D1 (+)		Cyclin D1 (-)		*P*
	n	%	n	%	
Age					
<40	11	84.6	2	15.4	.448
40-59	48	78.7	13	21.3	
>60	60	87.0	9	13.0	
Tumor size (cm)					
<2	21	75.0	7	25.0	.233
2-5	81	87.1	12	12.9	
>5	17	77.3	5	22.7	
Axillary lymph node metastasis					
Present	72	83.7	14	16.3	.843
Absent	47	82.5	10	17.5	
Multiple foci					
Present	19	79.2	5	20.8	.561
Absent	100	84.0	19	16.0	
Menopausal status					
Present	87	87.0	13	13.0	.075
Absent	28	73.7	10	26.3	
Chemotherapy					
Present	87	82.9	18	17.1	.404
Absent	29	90.6	3	9.4	
Radiotherapy					
Present	81	82.7	17	17.3	1.000
Absent	36	83.7	7	16.3	

intervals over an average period of 72 months. Among the 132 patients subject to follow-up, 48 succumbed to breast cancer, and 31 experienced a recurrence. The median overall survival time reached 147 months. Statistically, Cyclin D1 overexpression did not exhibit a significant impact on both OS and DFS (*P* = .189 and .06, respectively) (Figure 5). However, higher Cyclin D1 overexpression in ER-negative patients is associated with decreased DFS (*P* < .001), and similarly, it has a detrimental effect on OS and DFS in HER2-positive patients (Figure 6) (*P* = .042 and .026, respectively). Cyclin D1 overexpression had no significant effect on OS or DFS in ER-positive patients (*P* = .12 and .08, respectively).

DISCUSSION

Cyclin D1 expression in IBC patients via IHC reveal varying rates in the literature. In a recent study, Bouzidi et al. reported overall Cyclin D1 expression in IBC as 74%.[11] Our study on 143 patients showed 16.8% Cyclin D1-negative, 56.6% weakly positive, and 26.6% strongly positive, with an overall 83.2% overexpression rate, higher than reported in the literature (52-76.9%).[8,12,13] In interpreting these, findings it is crucial to consider the potential impact of threshold values and the specific Cyclin D1 clone used in the study.

Regarding clinical parameters, no significant correlation was observed between age, menopausal status, and Cyclin D1 overexpression in both the literature and our study.[8,11,12] Cyclin D1 expression and tumor size show conflicting results in the literature[11,14,15], but we found no significant correlation. Similar to our study, several publications do not report a correlation between Cyclin D1 and axillary lymph node metastasis[11,12,14], although He et al. suggest a significant and positive correlation.[15] Several studies, including ours, found no statistically significant correlation between clinical stage and Cyclin D1 expression.[8,11,12,14] However, He et al. reported a significant increase in Cyclin D1 expression among stage 1-2 patients.[15]

Due to the well-known roles of Cyclin D1 in cell migration, invasion, and metastasis[16], we aimed to investigate its potential association with parameters such as

Table 4. The relationship between Cyclin D1 and histological parameters of IBC cases

	Cyclin D1 (+)		Cyclin D1 (-)		*P*
	n	%	n	%	
Histologic type					
IBC of no special type (ductal)	103	83.0	21	17.0	.941
ILC	7	87.5	1	12.5	
Other*	9	81.8	2	18.2	
LVI					
Present	87	87.0	13	13.0	.820
Absent	28	73.7	10	26.3	
Histologic grade (NHS)					
Grade 1	14	87.5	2	12.5	.151
Grade 2	71	87.6	10	12.4	
Grade 3	32	74.4	11	25.6	
Extranodal extention					
Present	42	84.0	8	16.0	.934
Absent	30	83.3	6	16.7	
In situ component					
Present	21	75.0	7	25.0	1.000
Absent	81	87.1	12	12.9	
Stromal TIL					
Present	72	83.7	14	16.3	.534
Absent	47	82.5	10	17.5	
Intratumoral TIL					
Present	19	79.2	5	20.8	.371
Absent	100	84.0	19	16.0	

* The other histologic subgroups include invasive micropapillary carcinoma, metaplastic carcinoma, tubular carcinoma, and invasive papillary carcinoma. Abb. LVI: Lymphovascular invasion, NHS: Nottingham histologic score, IBC: Invasive breast carcinoma, ILC: Invasive lobular carcinoma, TIL: Tumor infiltrating lymphocytes.

Table 5. Correlation analysis of ER, PR, HER2, and Cyclin D1 in IBC cases (n=143)

	ER	PR	HER2	Cyclin D1
ER	1	0.550(**)	-0.289(*)	0.337(**)
PR	0.550(**)	1	-0.267(*)	0.318(**)
HER2	-0.289(*)	-0.267(*)	1	-0.083
Cyclin D1	0.337(**)	0.318(**)	-0.058	1

Pearson correlation coefficients are presented; (*) indicates significance at the 0.05 level, (**) indicates significance at the 0.01 level, and values without asterisks indicate non-significant correlations.

Table 6. Cyclin D1 expression status across different molecular subtypes of breast cancer

	Cyclin D1 (+)	Cyclin D1 (-)	*P*
Molecular Subtypes			
Luminal A	36	10	.008
Luminal B	65	5	
Triple-negative	9	6	
HER2	9	3	

HER2: Human Epidermal Growth Factor Receptor 2

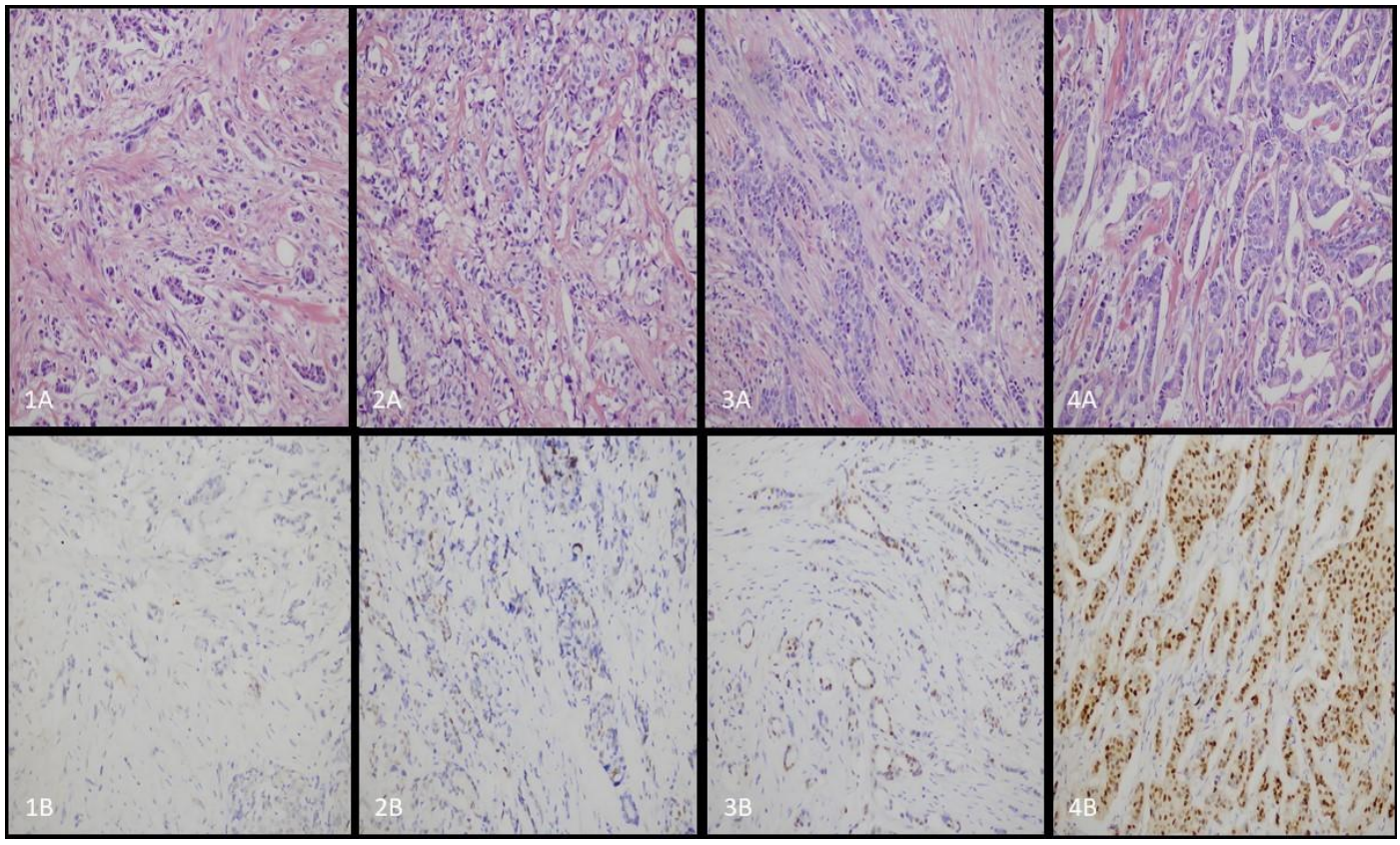

Figure 1-4. H&E and IHC images illustrating distinct Cyclin D1 expression levels in various IBC cases (H&E and IHC, x20 magnification for each image): Figure 1 shows invasive tumor (A) and Cyclin D1 negativity (B). Figure 2 shows invasive tumor (A) and score 1 Cyclin D1 positivity (B). Figure 3 shows invasive tumor (A) and 2 Cyclin D1 positivity (B). Figure 4 shows invasive tumor (A) and score 3 Cyclin D1 positivity (B).

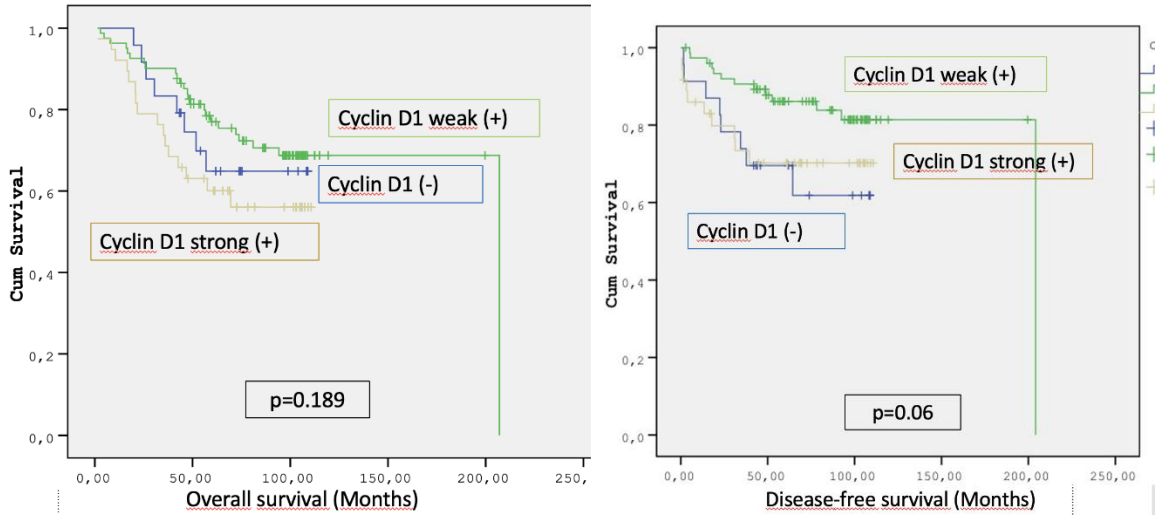

Figure 5. The correlation between Cyclin D1 expression and OS (A) and DFS (B) in unselected IBC cases.

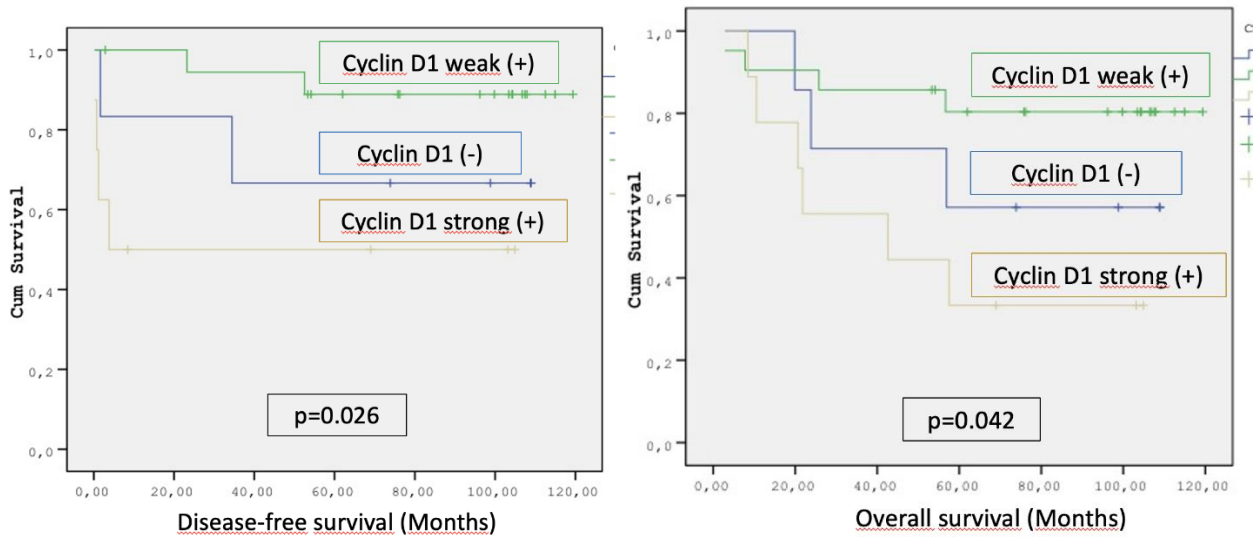

Figure 6. The correlation between Cyclin D1 expression and OS (A) and DFS (B) in HER2-positive IBC cases.

extranodal extension and LVI; however, no statistically significant correlation was found (P > .05). Similarly, the existing literature does not report any association between Cyclin D1 and LVI.[11] Studies on NHS (Nottingham Histologic Score) and Cyclin D1 relationship yielded mixed results[8,12,15]; our study found no significant correlation (P = .151). Cyclin D1's tightly regulated nature and diverse proliferation pathways in breast tumors may explain the lack of consistent correlation. No correlation was observed between the presence of carcinoma in situ and Cyclin D1, consistent with a recent study.[11] In our study, there was no observed association between Cyclin D1 and the presence of intratumoral and stromal TIL, recognized as a prognostic and predictive factor in IBC. To the best of our knowledge, there is no available data on this subject in the literature. Mylona et al. had linked Cyclin D1 overexpression to decreased p53 expression[12]; however, we observed no correlation between immunohistochemical p53 expression and Cyclin D1. This can be explained by differences in breast cancer populations from different geographic regions, tumor heterogeneity, and variations in IHC clones.

Numerous publications have consistently reported a positive correlation between overexpression of cyclin D1 and ER in breast cancer, and our study's results align with this pattern.[8,11,17] The direct interaction between Cyclin D1 and ER can activate nuclear receptors along with their coactivators, independent of CDK.[16] This CDK-independent nuclear receptor agonistic activity may also play a role in the oncogenic potential of Cyclin D1 in IBC.

HER2/*Neu* receptor amplification and Cyclin D1 overexpression are known to be associated with breast cancer. In HER2/*Neu*-induced breast tumors, the inhibitory effect of $p16^{INK4}$, a CDK 4 and 6 inhibitor, was demonstrated to block tumor formation in rats, thereby revealing an indirect link between HER2 and Cyclin D1.[18] Previous studies have indicated an increase in the expression of cell cycle pathway genes, such as CCND1 and CDK4, in HER2-enriched breast cancer.[19] In contrast to the findings of Guo et al. and Lee et al., our study did not identify a significant correlation between Cyclin D1 overexpression and HER2 (r= -0.083, P = .32). The number of HER2-positive cases in our sample was relatively limited (n=12), and divergent outcomes might be observed in more extensive datasets.

Considering molecular classes in our study, Luminal B group exhibited the highest Cyclin D1 expression, while the TNBC group showed the lowest. This finding is consistent with the known correlation between Cyclin D1 and ER and may reflect the estrogen-induced effects of this cell cycle regulator in IBC. Additionally, Guo et al. similarly found Luminal A group to have the highest Cyclin D1 expression.[8]

Conflicting findings have emerged regarding the impact of Cyclin D1 expression on OS and DFS in breast cancer. Some studies suggest an association with a favorable prognosis[8,20], while others indicate an unfavorable outcome.[8,21] In a meta-analysis of 21 breast cancer studies, Cyclin D1 gene amplification was linked to poor prognosis[22], while a 2020 meta-analysis by Binabaj et al. analyzing 34 studies found no prognostic effect of Cyclin D1 overexpression on breast cancer.[23] Furthermore, consistent with our study, certain publications propose that Cyclin D1 expression has no significant effect on prognosis.[11,24] Inconsistent results in the literature regarding prognosis and mortality may be associated with differences in Cyclin D1 measurement methods and cutoff selection, as well as

the heterogeneous nature of breast cancer. Focusing on the potential prognostic impact of Cyclin D1 in subgroups of breast cancer could provide further insights.

In ER-positive breast cancer, some studies propose an association between Cyclin D1 and a higher risk of mortality.[13,25-28] In a study parallel to ours, Cyclin D1 exhibited no impact on survival in ER-positive cancer.[29] One study examining the prognostic impact of cyclin D1 in ER-negative breast cancer identified it as a favorable factor[12], one as a negative factor[29], and other studies found no prognostic effect.[13,24,28] In our study, Cyclin D1 overexpression in ER-negative patients statistically significantly decreased DFS time ($P < .001$). ER-negative population is not homogenous and includes both HER2-positive and Triple-negative cases.

Cyclin D1 overexpression in HER2-positive patients is both related reduced DFS ($P = .026$) and OS time ($P = .042$) in our study. Research by Goel and colleagues revealed that Cyclin D1-CDK4 contributes to resistance in HER2+ breast cancer, with a crucial finding that this resistance was effectively overcome by combining CDK inhibitors and HER2-targeted therapy.[30] Despite the absence of a correlation between HER2 and Cyclin D1, the observed differences in survival times in our study could hold significant implications for the treatment of HER2-positive breast cancer. It has been suggested that the CCND1 gene may be linked to radiosensitivity in TNBC subgroup.[31] We did not find a significant relationship, but our sample size for TNBC cases was relatively small (n=15), and different results may be obtained in larger series.

Cyclin D1 has direct or indirect effects on treatment, especially in hormone-dependent breast carcinoma, and continues to be the subject of current studies. A combination of antiestrogen therapies, such as tamoxifen, along with CDK inhibitors, leads to cell cycle arrest specifically in the G1 phase.[32] The current NCCN guidelines recommend the combination of CDK 4/6 inhibitors with aromatase inhibitors as the first-line choice for a specific patient subgroup with hormone receptor-positive breast cancer.[7] Additionally, adiponectin-induced signals cause increased Cyclin D1 expression and breast tumor growth, positioning Cyclin D1 as a key target of adiponectin action in ER-positive breast cancer cells.[33] In recent years, there has been ongoing research into the role of miRNAs in the pathogenesis of IBC; however, clinical, and treatment-related implications have not yet been established. In Cyclin D1-induced breast cancer, activation of the miR-17/20 cluster is observed[34], and additionally, miR-21 and miR-93 trigger a pro-metastatic inflammatory response.[35]

Conclusively, we examined the relationship between Cyclin D1 expression and clinical-histopathological parameters in 143 cases diagnosed with IBC. A significant correlation was observed between ER and Cyclin D1. Cyclin D1 was overexpressed more in cases belonging to Luminal B group, while in Triple-negative group, it was less expressed. To the best of our knowledge, we compared the presence of stromal and intratumoral TIL, and extranodal extention with Cyclin D1 for the first time. Strong Cyclin D1 overexpression in HER2-positive cases significantly reduced OS and DFS.

Among the limitations of our study, it should be noted that our IBC cases represent a heterogeneous group. Furthermore, Cyclin D1 expression was evaluated exclusively at the protein level, without assessing mRNA, miRNA, or gene amplification. Similarly, HER2 status was determined solely by IHC, without FISH confirmation, which represents another limitation. Despite inconsistent findings in the literature regarding the potential prognostic impact of Cyclin D1, the effectiveness of CDK inhibitors in specific subgroups of breast cancer underscores the significance of conducting more extensive research in this area.

Etik Komite Onayı: Çalışmanın etik onayı 7 Haziran 2016 tarihinde İzmir Bozyaka Eğitim ve Araştırma Hastanesi Etik Kurulu'ndan alındı (Karar No. 4).
Hasta Onamı: Çalışma hakkında bilgi verildikten sonra tüm katılımcılardan yazılı bilgilendirilmiş onam alındı.
Hakem Değerlendirmesi: Dış bağımsız.
Yazar Katkıları: Fikir- EÇ; Tasarım-DKÇ; Denetleme-FT; Kaynaklar-FT; Veri Toplanması ve/veya İşlemesi-EV; Analiz ve/ veya Yorum- DKÇ; Literatür-AY; Yazıyı Yazan-EÇ: Eleştirel İnceleme- FT
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Ethics Committee Approval: Ethical approval for the study was obtained from the İzmir Bozyaka Education and Research Hospital Ethics Committee on June 7, 2016 (Decision No. 4).
Informed Consent: Written informed consent was obtained from all participants after providing information about the study.
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Author Contributions: Concept – EÇ; Design – DKÇ; Supervision – FT; Resources – FT; Data Collection and/or Processing – EV; Analysis and/or Interpretation – DKÇ; Literature Search – AY; Writing Manuscript – EÇ; Critical Review – FT
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REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185

Countries. *CA Cancer J Clin*. 2021;71(3):209-249. doi:10.3322/caac.21660
2. T.C. Saglik Bakanligi Halk Sagligi Genel Mudurlugu. Turkiye Kanser Istatistikleri 2018. Ankara: T.C. Saglik Bakanligi; 2022.
3. Tan PH, Ellis I, Allison K, et al. The 2019 World Health Organization classification of tumours of the breast. *Histopathology*.2020;77(2):181-185. doi:10.1111/his.14091
4. Roy M, Fowler AM, Ulaner GA, Mahajan A. Molecular Classification of Breast Cancer. *PET Clin.* 2023;18(4):441-458. doi:10.1016/j.cpet.2023.04.002
5. Tchakarska G, Sola B. The double dealing of cyclin D1. *Cell Cycle*. 2020;19(2):163-178. doi:10.1080/15384101.2019.1706903
6. Deshpande A, Sicinski P, Hinds PW. Cyclins and cdks in development and cancer: A perspective. *Oncogene*. 2005;24(17):2909-2915. doi:10.1038/sj.onc.1208618
7. Dwyer M. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®): Breast Cancer. NCCN.org. Published 2023. Available at: https://www.nccn.org.
8. Guo L, Liu S, Jakulin A, Yilamu D, Wang B, Yan J. Positive expression of cyclin D1 is an indicator for the evaluation of the prognosis of breast cancer. *Int J Clin Exp Med.* 2015;8(10):18656-18664.
9. Allison KH, Hammond MEH, Dowsett M, et al. Estrogen and progesterone receptor testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists guideline update. *Arch Pathol Lab Med.* 2020;144(5):545-563. doi:10.5858/arpa.2019-0904-SA
10. Wolff AC, McShane LM, Hammond MEH, et al. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *Arch Pathol Lab Med*. 2018;142(11). doi:10.5858/arpa.2018-0902-SA
11. Bouzidi L, Makni S, Feki J, et al. Immunohistochemical Overexpression of Cyclin D1 in Tunisian Invasive Breast Carcinoma Women. *Arch Iran Med*. 2022;25(4):250-256. doi:10.34172/aim.2022.41
12. Mylona E, Tzelepis K, Theohari I, Giannopoulou I, Papadimitriou C, Nakopoulou L. Cyclin D1 in invasive breast carcinoma: Favourable prognostic significance in unselected patients and within subgroups with an aggressive phenotype. *Histopathology*. 2013;62(3):472-480. doi:10.1111/his.12013
13. Ahlin C, Lundgren C, Embretsén-Varro E, Jirström K, Blomqvist C, Fjällskog ML. High expression of cyclin D1 is associated to high proliferation rate and increased risk of mortality in women with ER-positive but not in ER-negative breast cancers. *Breast Cancer Res Treat*. 2017;164(3):667-678. doi:10.1007/s10549-017-4294-5
14. Guo LL, Gao P, Wu YG, et al. Alteration of Cyclin D1 in Chinese Patients with Breast Carcinoma and its Correlation with Ki-67, pRb, and p53. *Arch Med Res*. 2007;38(8):846-852. doi:10.1016/j.arcmed.2007.06.004
15. He Y, Liu Z, Qiao C, Xu M, Yu J, Li G. Expression and significance of Wnt signaling components and their target genes in breast carcinoma. *Mol Med Rep*. 2014;9(1):137-143. doi:10.3892/mmr.2013.1774
16. Montalto FI, De Amicis F. Cyclin D1 in Cancer: A Molecular Connection for Cell Cycle Control, Adhesion and Invasion in Tumor and Stroma. *Cells*. 2020;9(12):2648. doi:10.3390/cells9122648
17. Ravikumar G, Ananthamurthy A. Cyclin D1 expression in ductal carcinoma of the breast and its correlation with other prognostic parameters. *J Cancer Res Ther.* 2014;10(3):671-675. doi:10.4103/0973-1482.138135
18. Yang C, Chen L, Li C, Lynch MC, Brisken C, Schmidt E V. Cyclin D1 enhances the response to estrogen and progesterone by regulating progesterone receptor expression. *Mol Cell Biol*. 2010;30(12):3111-3125. doi:10.1128/MCB.01398-09
19. The Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. *Nature*. 2012;490(7418):61-70. doi:10.1038/nature11412.
20. Holah NS, Hemida AS. Cyclin D1 and PSA act as good prognostic and clinicopathological indicators for breast cancer. *J Immunoassay Immunochem*. 2020;41(1):28-44. doi:10.1080/15321819.2019.1677706
21. Cheng CW, Liu YF, Yu JC, et al. Prognostic significance of cyclin D1, b-catenin, and MTA1 in patients with invasive ductal carcinoma of the breast. *Ann Surg Oncol*. 2012;19(13):4129-4139. doi:10.1245/s10434-012-2541-x
22. An HX, He Q, Wu J, et al. Clinicopathological and prognostic significance of cyclin D1 amplification in patients with breast cancer: a meta-analysis. *JBUON*. 2017;22(5):1209-1216.

23. Moradi Binabaj M, Bahrami A, Khazaei M, et al. The prognostic value of cyclin D1 expression in the survival of cancer patients: A meta-analysis. *Gene*. 2020;728:144283. doi:10.1016/j.gene.2019.144283
24. Lee A, Park WC, Yim HW, Lee MA, Park G, Lee KY. Expression of c-erbB2, cyclin D1 and estrogen receptor and their clinical implications in the invasive ductal carcinoma of the breast. *Jpn J Clin Oncol*. 2007;37(9):708-714. doi:10.1093/jjco/hym082
25. Bostner J, Ahnström Waltersson M, Fornander T, Skoog L, Nordenskjöld B, Stål O. Amplification of CCND1 and PAK1 as predictors of recurrence and tamoxifen resistance in postmenopausal breast cancer. *Oncogene*. 2007;26(49):6997-7005. doi:10.1038/sj.onc.1210506
26. Beca F, Pereira M, Cameselle-Teijeiro JF, Martins D, Schmitt F. Altered PPP2R2A and Cyclin D1 expression defines a subgroup of aggressive luminal-like breast cancer. *BMC Cancer*. 2015;15:285. doi:10.1186/s12885-015-1266-1.
27. Tobin NP, Lundgren KL, Conway C, Anagnostaki L, Costello S, Landberg G. Automated image analysis of cyclin D1 protein expression in invasive lobular breast carcinoma provides independent prognostic information. *Hum Pathol*. 2012;43(11). doi:10.1016/j.humpath.2012.02.015
28. Aaltonen K, Amini RM, Landberg G, Eerola H. Cyclin D1 expression is associated with poor prognostic features in estrogen receptor positive breast cancer. *Breast Cancer Res Treat*. 2008;113(1):75-82.
29. Umekita Y, Ohi Y, Sagara Y, Yoshida H. Overexpression of cyclinD1 predicts for poor prognosis in estrogen receptor-negative breast cancer patients. *Int J Cancer*. 2002;98(3):415-418. doi:10.1002/ijc.10151
30. Goel S, Wang Q, Watt AC, et al. Overcoming Therapeutic Resistance in HER2-Positive Breast Cancers with CDK4/6 Inhibitors. *Cancer Cell*. 2016;29(3):255-269. doi:10.1016/j.ccell.2016.02.006
31. Choi C, Park S, Cho WK, Choi DH. Cyclin D1 is associated with radiosensitivity of triple-negative breast cancer cells to proton beam irradiation. *Int J Mol Sci*. 2019;20(19):4943. doi:10.3390/ijms20194943
32. Pernas S, Tolaney SM, Winer EP, Goel S. CDK4/6 inhibition in breast cancer: current practice and future directions. *Ther Adv Med Oncol*. 2018;10:1758835918786451. doi:10.1177/1758835918786451.
33. De Amicis F, Chiodo C, Morelli C, et al. AIB1 sequestration by androgen receptor inhibits estrogen-dependent cyclin D1 expression in breast cancer cells. *BMC Cancer*. 2019;19(1):1038. doi:10.1186/s12885-019-6262-4.
34. Yu Z, Wang C, Wang M, et al. A cyclin D1/microRNA 17/20 regulatory feedback loop in control of breast cancer cell proliferation. *J Cell Biol*. 2008;182(3):509-517. doi:10.1083/jcb.200801079.
35. Lu J, Zhao Q, Ding X, et al. Cyclin D1 promotes secretion of pro-oncogenic immuno-miRNAs and piRNAs. *Clin Sci*. 2020;134(7): 791-805. doi:10.1042/CS20191318