The Breast 88 (2026) 104817

Contents lists available at ScienceDirect

The Breast

journal homepage: www.journals.elsevier.com/the-breast

Interplay between HER2-Low status, hormone receptor expression, and therapeutic response to cyclin-dependent kinase 4/6 inhibitors as first-line treatment in luminal-like metastatic breast cancer: The CYCLHER study

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ARTICLE INFO

Keywords:
HR expression
HER2-Low
CDK4/6 inhibitors
Advanced breast cancer
Real-world data

ABSTRACT

Background: In hormone receptor–positive/human epidermal growth factor receptor 2–negative (HR+/HER2−) metastatic breast cancer (mBC), the prognostic significance of estrogen receptor (ER), progesterone receptor (PgR), and HER2 immunohistochemical expression levels in patients treated with cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) remains unclear. The CYCLHER study was designed to evaluate treatment outcomes and identify biologically driven prognostic factors.

Methods: CYCLHER (NCT06243432) is a retrospective, multicenter study conducted across 16 Italian oncology centers. Patients with HR+/HER2− mBC who received first-line endocrine therapy (ET) plus CDK4/6i between November 2016 and May 2023 were included. ER, PgR and HER2 status were assessed on metastatic or primary

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https://doi.org/10.1016/j.breast.2026.104817
Received 22 February 2026; Received in revised form 11 May 2026; Accepted 16 May 2026
Available online 19 May 2026
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tumor samples. The primary endpoint was real-world progression-free survival (rwPFS). Secondary endpoints included overall survival (OS), objective response rate (ORR), disease control rate (DCR), duration of response (DoR), and attrition rate. Optimal cut-off values for ER and PgR expression were determined using Cut-off Finder v1.0.

Results: Among 597 eligible patients, median rwPFS was 28.1 months at a median follow-up of 41 months. HER2-low status emerged as a negative prognostic factor, being independently associated with shorter rwPFS compared to HER2-0 ($p = 0.02$). ER $\geq$ 87% and PgR $\geq$60% were associated with improved rwPFS, DCR, and 5-year OS rates. A prognostic score derived from the three biomarkers identified four groups with significantly different rwPFS outcomes (global log-rank $p < 0.0001$). The first-to-second line attrition rate was 16.2% (95% CI 12.4-20.0). The identified biomarkers did not significantly impact treatment outcomes in the second-line setting.

Conclusions: CYCLHER confirms the prognostic relevance of ER, PgR, and HER2 expression in patients with HR+/HER2−mBC treated with CDK4/6i. HER2-low status was associated with poorer outcomes, supporting its potential role as a negative prognostic marker. The proposed score may facilitate personalized treatment strategies and enhance risk stratification in clinical practice.

List of abbreviations

(ADCs) Antibody–drug conjugates
(AI) Aromatase inhibitor
(BC) Breast cancer
(CI) Confidence interval
(CDK4/6i) Cyclin-dependent kinase 4/6 inhibitors
(DCR) Disease control rate
(DoR) Duration of response
(ET) Endocrine therapy
(ER) Estrogen receptor
(HR) Hazard ratio
(HR+/HER2−) Hormone receptor–positive/human epidermal growth factor receptor 2–negative
(ILC) Invasive lobular carcinoma
(IHC) Immunohistochemistry
(ISH) In situ hybridization
(IDC) Invasive ductal carcinoma
(IQRs) Interquartile ranges
(LHRH) Luteinizing hormone-releasing hormone
(mBC) Metastatic breast cancer
(ORR) Objective response rate
(OS) Overall survival
(PgR) Progesterone receptor
(rwPFS) Real-world progression-free survival
(TTNT) Time to next treatment

1. Introduction

Breast cancer (BC) remains the most prevalent cancer diagnosis among women and a leading cause of cancer-related mortality worldwide [1]. Hormone receptor–positive/human epidermal growth factor receptor 2-negative (HR+/HER2−) BC is the most common subtype, accounting for approximately 70% of cases. Despite significant therapeutic advances, nearly one-third of patients diagnosed at early stages eventually relapse, which represents the majority of BC-related deaths [2].

In metastatic disease, the introduction of cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) combined with endocrine therapy (ET) dramatically improved survival outcomes of patients with HR+/HER2− metastatic breast cancer (mBC), both in endocrine-sensitive and endocrine-resistant settings [3–13]. Nevertheless, disease progression remains inevitable, and the identification of biomarkers predictive of sensitivity or resistance to CDK4/6i remains an unmet clinical need. On the other hand, although the addition of CDK4/6i to ET is generally more effective, a subset of patients may benefit from ET alone as first-line therapy, with the option of deferring CDK4/6i [14]. Genomic characterization is an emerging strategy for personalizing treatment [15], but limited access to comprehensive genomic testing worldwide, especially for first-line treatment, underscores the central role of immunohistochemical assessment of estrogen receptor (ER), progesterone receptor (PgR), and HER2 expression in guiding therapy.

Accumulating evidence indicates that semi-quantitative ER and PgR expression levels are independently associated with outcomes in patients treated with ET plus CDK4/6i [16,17]. However, results remain heterogeneous across series, partly due to methodological variability in receptor assessment, and the predictive value of ER and PgR has not yet been validated prospectively.

The heterogeneity of HER2 expression adds complexity. Approximately 60–65% of HR+/HER2– tumors are classified as HER2-low (immunohistochemistry [IHC]1+ or 2+ with in situ hybridization [ISH]-negative) [18]. Although current evidence does not support HER2-low as a distinct biological or clinical subtype, this phenotype has gained attention following the efficacy of novel antibody–drug conjugates (ADCs), most notably trastuzumab deruxtecan [19,20].

This scenario has been further complicated by the introduction of the HER2-ultralow category, defined as tumors with absent HER2 overexpression according to conventional criteria but showing faint/incomplete membrane staining in $\leq$10% of infiltrating tumor cells [21], and it represents a distinct therapeutic subgroup based on the result of the DESTINY-Breast06 trial [19] in HR+/HER2− mBC. However, to date, even more limited evidence is available regarding the prognostic or predictive role of HER2-ultralow status in patients receiving ET plus CDK4/6i, and its biological and clinical significance in this setting remains largely unexplored. Overall, the prognostic and predictive significance of both HER2-low and HER2-ultralow expression in the context of CDK4/6i plus ET remains controversial and unclear, as most available data derive from small, retrospective real-world series including patients treated across different lines of therapy [22–29]. In this context, the CYCLHER study leveraged real-world data to investigate the prognostic value of ER and PgR expression levels together with HER2 status in patients with HR+/HER2− mBC treated with first-line CDK4/6i plus ET. By integrating these routinely available biomarkers, we sought to develop a composite prognostic framework capable of refining risk stratification and potentially informing treatment strategies for patients with advanced HR+/HER2− disease.

2. Material and methods

2.1. Study design and patient population

The CYCLHER study (NCT06243432) is an observational,

retrospective, multicenter, cohort study conducted across 16 Italian oncology centers and coordinated by the Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome.

Eligible patients were aged ≥18 years at diagnosis and had histologically confirmed, locally advanced unresectable or metastatic HR+/HER2– BC. ER and PgR expression, as well as HER2 status assessed by IHC and ISH, were required in the pathology report of a metastatic biopsy, or, if not available, from the primary tumor. ER positivity was defined as ≥1% nuclear staining by IHC, in accordance with ASCO/CAP guidelines [30], with or without PgR positivity. HER2 negativity was defined as an IHC score of 0 or 1+, or 2+ with a negative ISH result [31], while HER2-low tumors were classified as IHC 1+ or 2+ with ISH negative. Patients must have received first-line ET with either an aromatase inhibitor (AI) or fulvestrant (plus a luteinizing hormone-releasing hormone [LHRH] agonist for premenopausal women), in combination with a CDK4/6i (palbociclib, ribociclib, or abemaciclib) as per clinical practice.

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board and Ethics Committee of the sponsor site (ID 5519). Clinical documentation was reviewed retrospectively, and informed consent was confirmed by direct contact with surviving patients.

2.2. Data collection

Data were extracted from patients' medical records and institutional databases, focusing on demographic information, tumor characteristics, treatment details, and follow-up outcomes. All data were annotated in a predefined study database. To ensure confidentiality, each patient was assigned a unique identification code by the study investigators. Participating centers shared the de-identified data with the coordinating site for centralized analysis. The dataset included variables such as age and sex at treatment initiation, histological and biological features of both primary and metastatic tumors, type of surgery, treatment regimens, duration and safety of therapies, and survival status at follow-up.

2.3. Study objectives and endpoints

The primary objective of this study was to assess the real-world effectiveness of first-line ET combined with CDK4/6i in subgroups of patients with HR+/HER2– mBC, according to ER, PgR and HER2-0/low expression.

The primary endpoint was real-world progression-free survival (rwPFS), defined as the time interval between the initiation of ET plus CDK4/6i and the first occurrence of disease progression or death from any cause according to ER, PgR and HER2-0/low expression. Disease progression was assessed locally, according to clinical practice. Patients who did not experience a rwPFS event were censored at the date of data cut-off or at the last follow-up visit, if it occurred earlier.

Secondary objectives included the evaluation of treatment activity and survival. Specifically, the secondary endpoints comprised: overall survival (OS), defined as the time from ET plus CDK4/6i initiation to death from any cause (for patients alive it was censored at the last follow-up); objective response rate (ORR), defined as the proportion of patients achieving a complete or partial response according to RECIST v1.1 criteria; disease control rate (DCR), defined as the proportion of patients achieving complete, partial response, or stable disease; duration of response (DoR), defined as the time from the first documented response to study treatment to disease progression or death. These endpoints were analyzed across subgroups defined by both receptor expression levels and treatment characteristics.

Exploratory real-world endpoints included: time to next treatment (TTNT), defined as the interval between initiation of ET plus CDK4/6i and the start of the next systemic therapy or death; the outcome of second-line treatments; and the prognostic impact of previously described biomarkers. The first-to-second-line attrition rate was also evaluated excluding patients with ongoing first line treatment. Patients were considered as experiencing attrition if they were not treated with second-line treatment and if they died within 6 months from the end of the first line [32].

2.4. Statistical analysis

Descriptive statistics were used to summarize baseline clinical and pathological characteristics. Categorical variables were reported as absolute frequencies and percentages, while continuous variables were expressed as medians and interquartile ranges (IQRs).

Survival outcomes, including rwPFS, DoR and OS, were estimated using the Kaplan-Meier method and, where applicable, compared across groups using the log-rank test. Cut-off Finder v1.0 was used to identify the optimal thresholds for ER and PgR expression, which maximize the differences between PFS curves in terms of log-rank test. Associations between baseline variables and rwPFS were assessed using univariable and multivariable Cox proportional hazards regression models, fitted for the following *a priori* defined (scientific approach) variables: menopausal status, estrogen and progesterone values, HER2 status, de novo metastatic diagnosis, sites of metastases, type of CDK4/6i and ET companion. Differences in categorical endpoints among the different subgroups, including ORR and DCR, were evaluated using the Chi-square test. Exploratory analyses were conducted to assess second-line treatment and its association with survival outcomes. All statistical tests were two-sided, and a p-value <0.05 was considered statistically significant; p-values were not adjusted for multiplicity. Analyses were performed using SPSS version 28.0 and R version 4.1.1, as appropriate.

2.5. Sample size

Based on the estimated enrollment capacity of participating centers, a total of 600 patients were expected to be included. Although no formal hypothesis testing was planned, this sample size was projected to yield approximately 240 events over a minimum follow-up of 18 months, allowing for the inclusion of up to 10 variables in multivariable models. A ratio of 25 events per variable was considered sufficient to ensure stable estimates and adequate statistical power [33].

3. Results

3.1. Patients' characteristics and ER/PgR thresholds

At the data cut-off a total of 618 patients were enrolled. Twenty-one patients not meeting the inclusion criteria were excluded from the analysis (Fig. S1). Therefore, 597 patients who initiated first-line treatment with CDK4/6i plus ET between November 2016 and May 2023 were included in the final analysis. The main clinical and pathological characteristics are summarized in Table 1. The median age at diagnosis was 58 years (IQR 49–67). The cohort was predominantly female (95.5%), with 79.4% of patients being post-menopausal. The most common histological subtypes were invasive ductal carcinoma (IDC) (66%) and invasive lobular carcinoma (ILC) (20.4%); 30.5% of patients had de novo metastatic disease. Visceral involvement was observed in 48.9% of patients, liver metastases were present in 21.4%, while 41.2% had bone-only disease; endocrine resistance was reported in 36.2% of the population [34].

Regarding treatment, palbociclib was the most frequently prescribed CDK4/6i (66.0%), followed by ribociclib (28.4%) and abemaciclib (5.7%). AIs were the most common ET backbone (64.7%), while fulvestrant was used in 35.3% of cases. Additionally, 20.6% of patients received a LHRH analogue (Table 1).

After a median follow-up of 41 months (IQR 30–53), 364 rwPFS events and 191 OS events were reported. The median rwPFS was 28.1 months (95% confidence interval [CI] 24.6–31.5), while the median OS was not reached.

Table 1
Patients' characteristics.

Baseline characteristics	Overall population n = 597
Age at diagnosis (median, IQR)-yr	58 (49–67)
Gender – n (%)	
Female	594 (95.5)
Male	3 (0.5)
Menopausal status - n (%)	
Pre-menopausal	123 (20.6%)
Post-menopausal	474 (79.4%)
ECOG PS - n(%)	
0	423 (70.9%)
1	147 (24.6)
2	21 (3.5)
NA	6 (1.0)
Histology - n (%)	
IDC	394 (66.0)
ILC	122 (20.4)
Mixed	9 (1.5)
Other	27 (4.5)
NA	45 (7.5)
ER expression (median, IQR)	90 (80–95)
ER expression categories, n (%)	
ER ≥ 80	513 (86.0)
ER 50-79	54 (9.0)
ER 10-49	25 (4.2)
ER < 10	5 (0.8)
PgR expression (median, IQR)	60 (20–90)
HER2 status- n (%)	
0	254 (42.5)
1+	207 (34.7)
2+ ISH not amplified	136 (22.8)
De novo metastatic disease - n (%)	
Yes	182 (30.5)
No	415 (69.5)
Metastasis - n (%)	
Visceral	292 (48.9)
Non-Visceral	305 (51.1)
Prior Chemotherapy - n (%)	
Neoadjuvant	90 (15.1)
Adjuvant	203 (34.0)
Prior adjuvant ET - n (%)	
Yes	373 (89.9)
No	42 (10.1)
Endocrine resistance	
Yes	216 (36.2)
No	381 (63.8)
CDK4/6 inhibitor - n (%)	
Palbociclib	394 (66.0)
Ribociclib	170 (28.4)
Abemaciclib	33 (5.7)
ET first line companion - n(%)[a]	
AI	386 (64.7)
Fulvestrant	211 (35.3)

DFI, disease free interval; ECOG PS, Eastern Cooperative Oncology Group performance status; ET, endocrine therapy; HER2, human epidermal growth factor receptor 2; IQR, Interquartile range; IDC, Invasive ductal carcinoma; ILC, invasive lobular carcinoma; ISH, hybridization in situ; LHRH analogue, Luteinizing Hormone-Releasing Hormone analogue; N, number; NA, not available.

[a] *Fifty patients received fulvestrant in an endocrine-sensitive setting, whereas 44 received an AI for endocrine-resistant disease, according to clinical choice.*

When considered as continuous variables, the optimal cut-off values were 87% for ER (Hazard ratio [HR] for rwPFS: 0.99; 95% CI 0.99–1.00; p = 0.025) and 60% for PgR (HR for rwPFS: 0.99; 95% CI 0.99–1.00; p < 0.001).

3.2. rwPFS according to ER, PgR and HER2 expression

Stratifying the population according to these cut-offs, patients with high ER expression (≥87%) had a significantly longer median rwPFS compared to those with low ER expression (<87%) (32.4 vs. 21.4 months; HR 0.70; 95% CI 0.56–0.88; p = 0.002). Similarly, high PgR expression (≥60%) was associated with improved rwPFS compared to low PgR expression (<60%) (40.9 vs. 22.6 months; HR 0.59; 95% CI 0.47–0.73; p < 0.001) (Fig. 1a and b).

Patients with HER2-low disease had a median rwPFS of 26.6 months compared to 30.8 months in the HER2-0 group (HR 1.26; 95% CI 1.02–1.55; p = 0.033) (Fig. 1c); the median rwPFS decreased with increasing HER2 expression (median rwPFS: HER2-0, 30.8 months [95% CI 24.1–37.4]; HER2-1+, 27.3 months [95% CI 21.9–32.7]; HER2-2+, 23.0 months [95% CI 15.5–30.6]).

In multivariable Cox regression models adjusted for clinical and therapeutic characteristics, the independent prognostic value for rwPFS of the identified cut-off for ER ≥ 87% (HR 0.74; 95% CI 0.58–0.92) and PgR ≥60% (HR 0.68; 95% CI 0.55–0.84), as well as HER2-low status (HR 1.28; 95% CI 1.04–1.59) was confirmed (Table 2).

Interestingly, no significant impact of the type of CDK4/6i prescribed was observed in the multivariable model; while Fulvestrant as ET companion was associated with worse outcome (HR 1.49; 95 % CI 1.17–1.90) (Table 2).

Based on this result, we developed a prognostic score based on three biological variables: ER expression ≥87%, PgR expression ≥60%, and HER2-0 status. Patients were categorized into four groups according to the number of favorable factors present: score 0 (no favorable factors), score 1 (one favorable factor), score 2 (two favorable factors), and score 3 (all three favorable factors). Kaplan-Meier analysis revealed a clear stratification of rwPFS across the four groups, with a stepwise improvement in outcomes as the number of favorable features increased (median rwPFS for score 0: 14.8 months [95% CI 10.2-19.3], for score 1: 22.8 months [95% CI 18.9-26.7], for score 2: 32.4 months [95% CI 25.9-38.9], for score 3: 53.2 months [95% CI 41.0-65.4]; global log-rank test: p < 0.0001) (Fig. 2).

3.3. Secondary endpoints according to ER, PgR and HER2 expression

An ORR of 44.7% was observed in the overall cohort and was higher in patients with ER ≥ 87% compared to those with ER <87% (47.6% vs. 37.0%); in contrast, median DoR was longer in patients with PgR ≥60% (60.1 vs. 31.4 months). A higher DCR was observed in subgroups above the ER and PgR thresholds (86.8% vs. 78.6% for ER; 89.8% vs. 80.1% for PgR). The 5-year OS rate was also different among these subgroups. A clinically relevant difference in 5-year OS was observed between HER2-0 and HER2-low subgroups (58.0% vs. 48.2%) (Table 3).

3.4. Exploratory analysis on second line treatment

The median TTNT was 29.5 months (95% CI 26.1-33.3). Among the 364 patients evaluable for the analysis, the first-to-second-line attrition rate was 16.2% (95% CI 12.4-20.0), with no differences according to ER, PgR and HER2 expression.

Among the entire cohort, 51.1% of patients (n = 305) had received a second-line treatment, of whom 56.4% received a chemotherapy regimen. A total of 19% were treated with the combination of an AI (mostly exemestane) plus everolimus while 19% received an ET± a targeted therapy; 5.6% received a treatment in a clinical trial. At a median follow-up of 22 months calculated from the second-line initiation, 126 rwPFS events were reported. The median rwPFS for second-line treatment was 6.1 months (95% CI 5.2–7.0).

A multivariable analysis was then performed to assess whether second-line treatment type, ER and PgR expression (based on previously identified thresholds), and HER2 status affected outcomes. None of the variables included in the model showed statistically significant associations with second-line rwPFS (Table S1).

4. Discussion

The CYCLHER study contributes to the growing real-world evidence on the use of CDK4/6i in HR+/HER2– mBC, with a particular focus on the prognostic role of HR and HER2 expression. The rwPFS with first-

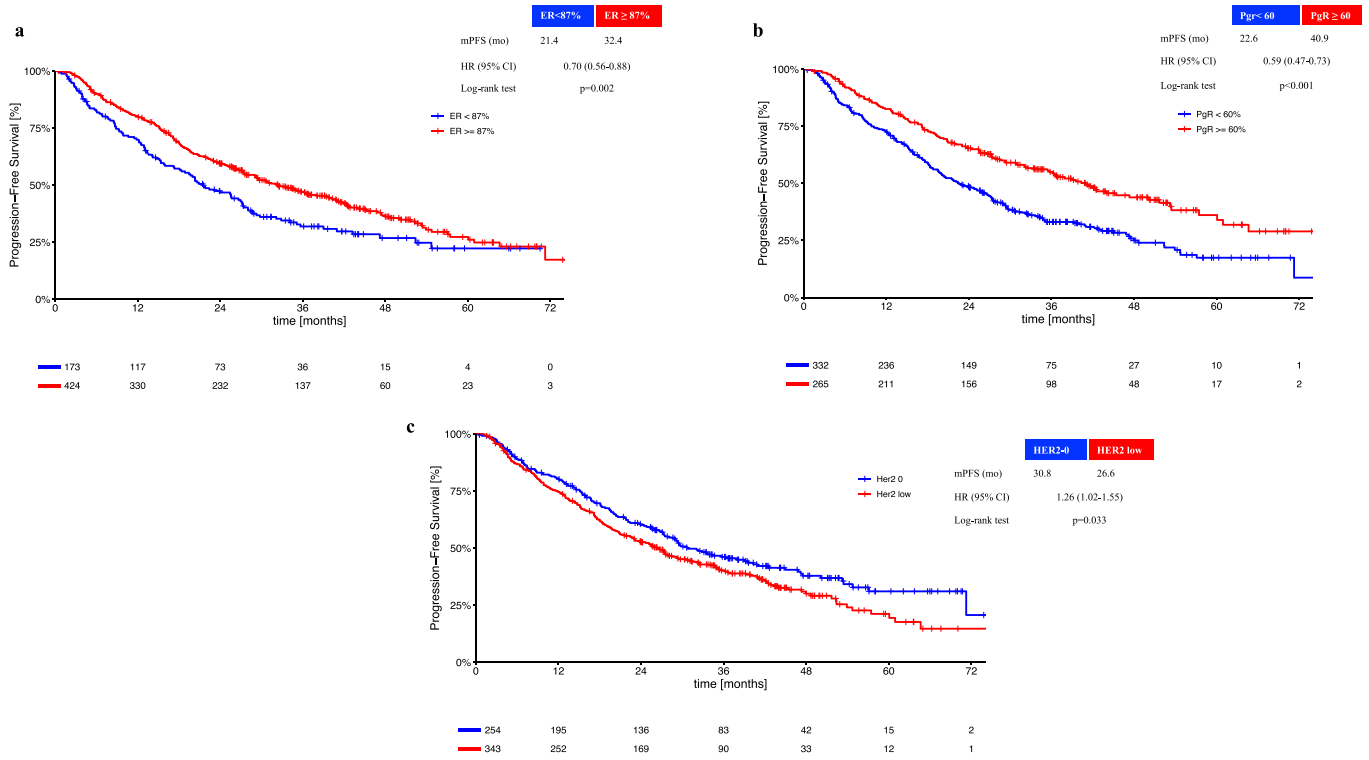


Fig. 1. Kaplan–Meier curves of rwPFS according to ER expression (a), PgR expression (b), and HER2 status (c).
Patients were stratified using biomarker-specific cut-offs identified via Cut-off Finder for ER and PgR expression. Survival differences were assessed with log-rank tests and Cox models. *rwPFS = real-world Progression-Free Survival; ER = Estrogen Receptor; PgR = Progesteron Receptor; HER2 = Human Epidermal Growth Factor Receptor 2.*

line CDK4/6i plus ET was affected by high expression levels of ER and PgR, with cut-offs identified at 87% and 60%, respectively. The clinical relevance of ER and PgR levels has been previously explored in the PALOMA-3 trial by Cristofanilli et al. [17], where PgR H-scores were found to be consistently higher among long-term responders, regardless of treatment arm, suggesting a potential link between PgR expression and endocrine sensitivity. Further support comes from an exploratory analysis of the PADA-1 trial which identified ER and PgR expression as independent prognostic factors for PFS in patients receiving first-line palbociclib plus aromatase inhibitors. Notably, even patients with low ER expression (<50%) achieved meaningful benefit, reinforcing the broad efficacy of CDK4/6i across receptor levels [16]. Our findings, that take into account also other CDK4/6i, align with these observations and suggest that quantitative receptor assessment may help refine prognostic stratification. However, the interpretation of ER as a continuous biomarker remains complex, particularly at the lower end of its expression spectrum. The focus on ER-low breast cancer (defined as ER expression in 1–10% of tumor cells) has gained increasing attention in recent years[35,36]. These tumors represent a biologically heterogeneous subgroup between classical ER-positive and triple-negative breast cancer, with a less clear benefit from ET[37,38]. In our study, patients with ER-low tumors were included according to current ASCO/CAP guidelines (≥1%) [30]; however, their representation was limited (n = 5), precluding dedicated analyses and possibly reflecting their different management in clinical practice.

Interestingly, even patients with biomarker expression below the identified cut-offs achieved median rwPFS values comparable to those reported in registrational trials; however, the absence of genomic profiling in our cohort limits the ability to define predictive signatures, and further molecular characterization is warranted. The impact of ER and PgR expression on secondary endpoints was less clear, although both biomarkers significantly influenced DCR. A better genomic definition could justify these results, given that there are no clinical correlations to emphasize.

HER2-low status, increasingly recognized as a clinically relevant subgroup, emerged in our cohort as being associated with less favorable outcomes. This is consistent with the retrospective multicenter study by Zattarin et al. which reported significantly shorter PFS and OS in HER2-low patients treated with CDK4/6i plus ET [29]. Although median OS was not reached in our cohort, a clinically relevant difference in 5-year OS was observed. While previous studies have explored this association, many were limited by small sample sizes or inclusion of patients treated in different lines [25,39,40]. A secondary analysis of PALOMA-2 and PALOMA-3 by Li et al. further suggested differential outcomes based on HER2 expression, with HER2-low patients deriving benefit from palbociclib plus ET, while HER2-0 patients responded primarily if previously exposed to ET [41]. More recently, the introduction of the HER2-ultralow category has further expanded the spectrum of HER2-negative disease. Based on the results of the DESTINY-Breast06 trial [19], HER2-ultralow has been recognized as a distinct therapeutic subgroup [42], given the activity of trastuzumab deruxtecan also in these patients. However, from a biological and prognostic perspective, current evidence does not support HER2-ultralow as a clearly distinct entity from HER2-null disease. Recent molecular analyses from the PENELOPE-B cohort showed no significant differences in survival outcomes or HER2 mRNA expression between HER2-ultralow and HER2-null tumors, while HER2-low appeared as a more clearly defined biological subgroup [43]. Similarly, retrospective studies reported no significant prognostic differences between HER2-ultralow and HER2-null disease in HR+/HER2 BC [44–46].

The lack of significance of HER2-low status on secondary endpoints in our analysis, combined with the retrospective nature of the study and the heterogeneity of available evidence highlights the need for further investigation in larger, prospective studies.

Table 2
Multivariable Cox Regression analysis in term of rwPFS.

Clinical variables	*Univariable*		*Multivariable*	
	Hazard Ratio (95%CI)	*P* value	Hazard Ratio (95%CI)	*P* value
Post-menopausal *vs* pre-menopausal	0.94 (0.73-1.21)	0.65	0.90 (0.70-1.18)	0.46
ER ≥ 87% *vs.* <87%	0.70 (0.56–0.88)	**0.002**	0.74 (0.58–0.92)	**0.009**
PgR ≥60 *vs.* <60%	0.59 (0.47–0.73)	**<0.001**	0.68 (0.55–0.84)	**<0.001**
HER2-low *vs.* HER2-0	1.26 (1.02–1.55)	**0.033**	1.28 (1.04-1.59)	**0.02**
De novo metastatic disease *vs.* recurrent disease	0.62 (0.49–0.78)	**<0.001**	0.82 (0.63–1.08)	0.16
Endocrine-resistant *vs.* endocrine-sensitive disease	1.84 (1.49–2.27)	**<0.001**	1.38 (1.00–1.91)	0.05
Metastatic site		**0.003**		0.49
Visceral *vs.* non-visceral	1.63 (1.10-2.42)		1.26 (0.84-1.91)	
Bone-only *vs.* non visceral	1.19 (0.79-1.79)		1.28 (0.84-1.96)	
Liver metastases vs no liver metastases	1.88 (1.49-2.37)	**<0.001**	1.75 (1.31-2.34)	**<0.001**
CDK4/6 inhibitor		0.11		0.31
Ribociclib vs. palbociclib	0.85 (0.67–1.08)		0.95 (0.74–1.22)	
Abemaciclib vs. palbociclib	1.39 (0.88–2.19)		1.38 (0.87–2.18)	
Fulvestrant vs. AI	1.76 (1.43–2.18)	**<0.001**	1.49 (1.17–1.90)	**0.001**

Multivariable Cox regression analysis evaluating the association between clinical and therapeutic variables and rwPFS. HR are adjusted for all listed covariates. A global p-value is reported for the CDK4/6 inhibitor variable, with Palbociclib used as the reference category.
rwPFS, real-world progression-free survival; HR, hazard ratio; CI, confidence interval; ER, estrogen receptor; PgR, progesterone receptor; HER2, human epidermal growth factor receptor 2; AI, aromatase inhibitor.

By combining these three independent prognostic factors, i.e., ER expression ≥87%, PgR expression ≥60%, and HER2-0 status, we developed a composite score reflecting the number of favorable biological features present. Patients were categorized into four groups (score 0–3), and the Kaplan-Meier analysis revealed a progressive improvement in rwPFS with each additional favorable factor. This finding underscores the cumulative prognostic value of routine immunohistochemical markers and suggests that their integration into a simple scoring system may help identify patients with more indolent disease biology. Conversely, patients lacking all favorable features (score 0) experienced the poorest outcomes, highlighting a subgroup with potentially more aggressive disease. Although the prospective validation of the thresholds identified for the biomarkers expression is needed.

This observation reinforces the need for integrated biomarker strategies that go beyond standard immunohistochemistry analysis. Indeed, the interplay between histochemical markers and intrinsic molecular subtypes remains a critical area of investigation in HR+/HER2– disease [47]. Standard IHC may not fully capture the biological complexity of HR+/HER2− disease. The ongoing PONTIAC trial (NCT06486883) is addressing this gap by evaluating trastuzumab-deruxtecan versus CDK4/6i-based ET in patients classified as non-luminal by PAM50 profiling or with ER <50%, potentially paving the way for more personalized treatment approaches.

The absence of significant differences among the three CDK4/6i in our multivariable model is consistent with the P-VERIFY study, which reported comparable real-world PFS across agents in U.S. clinical practice [48]. However, our study was not powered for direct comparisons, and imbalance among the three CDK4/6i was influenced by availability and reimbursement policies in our country.

In our cohort, the first-to-second-line attrition rate was 16.2% (95% CI 12.4-20.0). This rate is higher than the 8.5% (95% CI 7.2% −9.9%) reported for HR+/HER2– patients in the large Italian GIM14/BIO-META cohort [32]. Notably, Blondeaux et al. also observed higher attrition among patients treated with CDK4/6i plus ET compared to other regimens, suggesting that progression after prolonged disease control may be more clinically aggressive and less manageable. These findings support the interpretation of attrition not merely as a failure to transition to second-line therapy, but as a surrogate marker of disease biology and progression dynamics.

Second-line treatment patterns in our cohort, due to the time of start enrollment, do not reflect current guideline recommendations and clinical practice [49]. Chemotherapy was the predominant option, while the use of targeted therapies such as everolimus-exemestane or ET-based

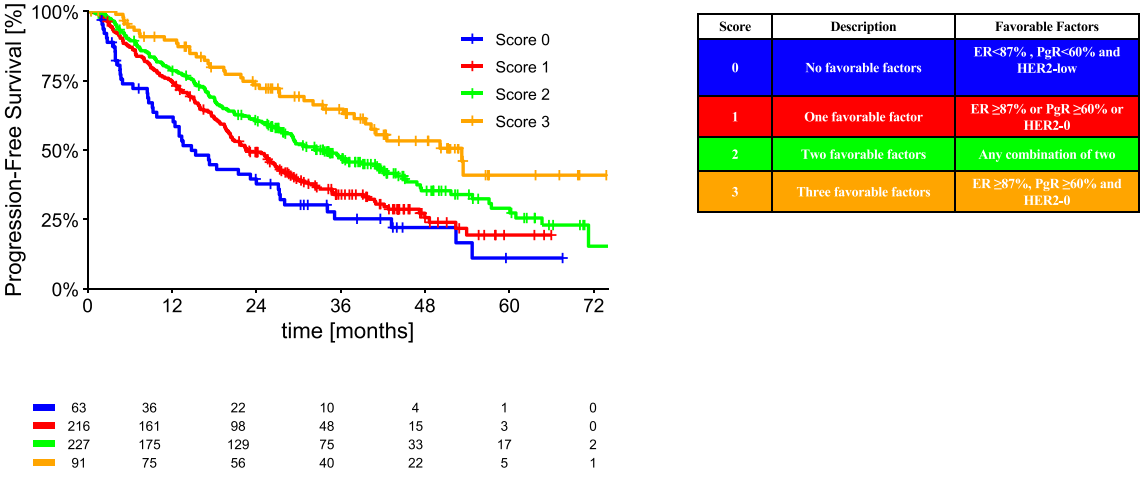


Fig. 2. Kaplan-Meier curves of rwPFS according to prognostic score based on ER, PgR, and HER2-low status. Patients were grouped by the number of favorable biological factors: ER ≥ 87%, PgR ≥60%, and HER2-0. A progressive improvement in PFS was observed with increasing score. The global log-rank test was significant ($p < 0.0001$), and all pairwise comparisons except score 0 vs 1 ($p = 0.068$) were statistically significant. *ER, Estrogen Receptor; HER2-low, Human Epidermal Growth Factor Receptor 2 with IHC score 1+ or 2+ and negative ISH; IHC, Immunohistochemistry; ISH, In Situ Hybridization; rwPFS = real world Progression-Free Survival; PgR, Progesterone Receptor.*

Table 3
Secondary endpoints according to ER, PgR and HER2 expression.

	All pts	ER < 87	ER ≥ 87	P	PgR<60	PgR≥ 60	P value	HER2-0	HER2-low	P value
ORR –N (%)	267 (44.7)	64 (37.0)	203 (47.6)	**0.015**	140 (42.2)	127 (47.9)	0.16	107 (42.1)	160 (46.6)	0.27
DCR-N (%)	504 (84.4)	136 (78.6)	368 (86.8)	**0.012**	266 (80.1)	238 (89.8)	**0.001**	220 (86.6)	284 (82.8)	0.20
DoR-Median (95%CI)	43.9 (33.2-54.7)	35.1 (23.5-46.7)	50.1 (38.2-62.0)	0.17	31.4 (25.5–37.3)	60.1 (48.2–71.9)	**<0.001**	45.0 (29.1–60.8)	41.9 (27.4–56.4)	0.72
5-y OS (%)	52.3	45.9	54.8	**0.027**	44.9	61.6	**0.003**	58.0	48.2	0.058

ORR, DCR, and 5-year OS were compared across subgroups defined by ER, PgR, and HER2 status. Median DoR was also reported. Differences in ORR, DCR, and OS rates were assessed using the chi-square test, while DoR and OS comparisons were performed using the log-rank test.
ORR, objective response rate; DCR, disease control rate; DoR, duration of response; 5y OS, 5 years overall survival; ER, estrogen receptor; PgR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

regimens was limited. This may explain the lack of predictive value of ER, PgR and HER2 expression in this setting. Moreover, none of the patients received ADCs as subsequent lines.

There are limitations to this study that should be acknowledged. First, its retrospective design inherently introduces potential biases, including selection and incomplete data capture. Second, the long enrollment period (2016–2023) may have led to heterogeneity in clinical practice, drug availability, diagnostic and therapeutic standards; however, it also allowed for a comprehensive overview of the evolving real-world management of HR+/HER2− mBC. Third, the lack of centralized pathology review and genomic profiling limits the precision of biomarker stratification and prevents deeper insights into intrinsic subtypes. In particular, HER2-ultralow status could not be reliably assessed, as this category was not routinely reported in pathological records due to the time in which the study treatment was carried out and no central pathology review was available. Therefore, a separate analysis of HER2-ultralow disease, which may represent an emerging therapeutic subgroup despite lacking a clearly distinct biological profile from HER2-null tumors, could not be performed. In addition, the absence of comprehensive genomic profiling (including alterations such as PIK3CA mutations) further limits the ability to integrate molecular data with immunohistochemical findings and to place our results within the current biomarker-driven therapeutic landscape [50]. Fourth, the absence of standardized criteria for second-line treatment selection, characterized by a predominance of chemotherapy and limited use of targeted agents, may have affected the interpretation of post–CDK4/6i outcomes. This limitation is further compounded by the lack of genomic profiling, which currently plays a key role in guiding treatment decisions in later lines of therapy. Finally, the study was not powered for direct comparisons between CDK4/6i, and the observed equivalence should be interpreted with caution; a more homogeneous comparison between the three CDK4/6i should be needed. On the other hand, CYCLHER represents one of the largest real-world cohorts integrating clinical and biological data, with a relatively uniform therapeutic approach among participating centers. Despite these limitations, the multicenter design and the inclusion of both high-volume referral institutions and lowest patient volume centers enhance the representativeness of real-world clinical practice and support the generalizability of our findings. Moreover, the sample size was consistent with the planned statistical design of the study, supporting the reliability of the primary analyses, while subgroup and exploratory biomarker-based analyses should be interpreted with appropriate caution.

In conclusion, the CYCLHER study provides real-world evidence supporting the prognostic relevance of ER, PgR, and HER2-low expression in HR+/HER2– mBC treated with CDK4/6i plus ET. Our findings suggest that HER2-low status may identify a subgroup with less favorable disease biology even within the HR+/HER2− spectrum. However, the proposed biomarker thresholds and the composite prognostic score should be considered hypothesis-generating rather than immediately applicable to routine clinical practice, and prospective validation in larger and more homogeneous cohorts is required before clinical implementation. These results underscore the need for refined, biomarker-driven stratification and support the integration of quantitative histochemical assessment with genomic profiling in future prospective studies to optimize treatment personalization.

CRediT authorship contribution statement

Letizia Pontolillo: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Elisabetta Munzone:** Writing – review & editing, Data curation. **Paolo Vigneri:** Writing – review & editing, Data curation. **Mirco Pistelli:** Writing – review & editing, Data curation. **Antonella Palazzo:** Writing – review & editing, Data curation. **Nicla La Verde:** Writing – review & editing, Data curation. **Marco Mazzotta:** Writing – review & editing, Data curation. **Ida Paris:** Writing – review & editing, Data curation. **Daniele Alesini:** Writing – review & editing, Data curation. **Daniele Santini:** Writing – review & editing, Data curation. **Katia Cannita:** Writing – review & editing, Data curation. **Armando Orlandi:** Writing – review & editing, Data curation. **Emanuela Magnolfi:** Writing – review & editing, Data curation. **Laura Giacinti:** Writing – review & editing, Data curation. **Mimma Raffaele:** Writing – review & editing, Data curation. **Luisa Carbognin:** Writing – review & editing, Data curation. **Angela Santoro:** Writing – review & editing, Data curation. **Alessandro Rossi:** Writing – review & editing, Data curation. **Giorgia Arcuri:** Writing – review & editing, Data curation. **Claudia Sangalli:** Writing – review & editing, Data curation. **Ornella Garrone:** Writing – review & editing, Data curation. **Antonio Mulè:** Writing – review & editing. **Carmine Valenza:** Writing – review & editing, Data curation. **Rossana Berardi:** Writing – review & editing, Data curation. **Giampaolo Tortora:** Writing – review & editing, Data curation. **Emilio Bria:** Writing – review & editing, Data curation. **Giuseppe Curigliano:** Writing – review & editing, Data curation. **Diana Giannarelli:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Alessandra Fabi:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization.

Data availability statement

The data generated and/or analyzed during the current study are available from the corresponding author upon a reasonable request.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board and Ethics Committee of the sponsor site (ID 5519).

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT in order to assist with grammar checking and to improve readability in

sections where the writing could have been unclear. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Funding sources

This study was funded by the Italian minister of health – Ricerca Corrente 2024.

Conflicts of interest

LP reports travel grants from Pfizer, Eli Lilly and Gilead; speaker honoraria from Daiichi Sankyo and Novartis; and consulting fees from Menarini Silicon Biosystems. LP is a Young Committee Member of the International Society of Liquid Biopsy (ISLB). All outside the submitted work.

EM reports consulting/advisory fees from Eisai, Exact Sciences, MSD Oncology, Daiichi Sankyo/AstraZeneca, Pfizer and Seagen. All outside the submitted work.

PV reports advisory roles and honoraria from AstraZeneca, Daiichi Sankyo, Eli Lilly, Gilead, Incyte, Istituto Gentili, Novartis, Pfizer, Roche, Seagen and Teva; travel and accommodation support from Daiichi Sankyo, Eli Lilly and Novartis; and research funding to the institution from Novartis and AstraZeneca. All outside the submitted work.

MP reports consulting/advisory fees from AstraZeneca, Eli Lilly, Gilead, Novartis, Pfizer and Daiichi Sankyo, and travel support from Roche and MSD. All outside the submitted work.

AP reports consulting/advisory fees from Amgen, MSD and Novartis, and travel and accommodation support from Pfizer. All outside the submitted work.

NLV reports consulting/advisory roles from Novartis, Pfizer, Roche, MSD, AstraZeneca and Eisai; speaker honoraria from Pfizer, Roche, Gentili, Eli Lilly, Gilead, Daiichi Sankyo and Techdow; travel support from Pfizer and Roche; and research grants from GSK and Gilead. All outside the submitted work.

IP reports advisory and speaker honoraria from Novartis, Pfizer, AstraZeneca, Seagen, MSD, Eli Lilly, Gilead, Menarini Stemline, Gentili, Daiichi Sankyo, Genetic and Italfarmaco. All outside the submitted work.

DS reports advisory roles for Amgen, Janssen, Astellas, Bayer, Servier, Novartis and MSD. All outside the submitted work.

AO reports consulting/advisory fees from Novartis, Roche, Eli Lilly, Amgen and Daiichi Sankyo, and travel and accommodation support from Daiichi Sankyo, Novartis, Roche and Pfizer. All outside the submitted work.

LC reports speaker honoraria from Daiichi Sankyo, Novartis, AstraZeneca and Menarini. All outside the submitted work.

GT reports research funding from Ministero della Salute (Ricerca Corrente 2022), AIRC (IG26330), Ministero dell'Università e della Ricerca (PRIN 2022 PNRR Prot P2022LN3KS; PRIN 2022 Prot 2022P79F9N), and Agenzia Italiana del Farmaco–Ministero della Salute (J38D19000690001 FIMP); and consulting/advisory roles for Bristol Myers Squibb, AstraZeneca, MSD, Merck and Servier. All outside the submitted work.

EB reports institutional research grants from AstraZeneca and Roche; speaker honoraria from Merck Sharp & Dohme, AstraZeneca, Pfizer, Eli Lilly, Bristol Myers Squibb, Novartis, Takeda and Roche; and consulting/advisory fees from Merck Sharp & Dohme, Pfizer, Novartis, Bristol Myers Squibb, AstraZeneca, Roche, Amgen and Celltrion. All outside the submitted work.

GC reports research grants from Merck; honoraria from Ellipses Pharma; travel support from Roche/Genentech, Pfizer, Daiichi Sankyo and AstraZeneca; leadership roles in ESMO, the European Society of Breast Cancer Specialists and *ESMO Open*; speaker honoraria from Roche/Genentech, Novartis, Pfizer, Eli Lilly, Foundation Medicine, Samsung, Daiichi Sankyo, Seagen, Menarini, Gilead Sciences, AstraZeneca and Exact Sciences; and consulting/advisory roles for Roche/Genentech, Pfizer, Novartis, Eli Lilly, Foundation Medicine, Bristol Myers Squibb, AstraZeneca, Daiichi Sankyo, GlaxoSmithKline, Seagen, Guardant Health, Veracyte, Celcuity, Menarini, Merck, Exact Sciences, Blueprint Medicines and Gilead Sciences. All outside the submitted work.

AF reports consulting/advisory roles for Roche, Eli Lilly, Novartis, AstraZeneca, Pfizer, Seagen, Gilead Sciences, MSD, Menarini Stemline and Dompé Biotech; speaker honoraria from AstraZeneca, Roche, Eli Lilly, Novartis, Gilead Sciences, Pfizer, Daiichi Sankyo and Exact Sciences; research support from AstraZeneca and Roche. All outside the submitted work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.breast.2026.104817.

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