

# Baseline lymphopenia as prognostic factor in patients with metastatic breast cancer treated with palbociclib

GEORGE EMILE<sup>1</sup>, SARAH PENAGER<sup>2</sup>, CHRISTELLE LEVY<sup>1</sup>, ALISON JOHNSON<sup>1</sup>, DJELILA ALLOUACHE<sup>1</sup>, JUSTINE LEQUESNE<sup>2</sup>, IOANA HRAB<sup>1</sup>, CARINE SEGURA<sup>1</sup>, ADELINE MOREL<sup>1</sup>, KATARINA GUNZER<sup>1</sup>, AUDREY FAVEYRIAL<sup>1</sup>, FRANCOIS CHERIFI<sup>1</sup> and ANGELIQUE DA SILVA<sup>1</sup>

<sup>1</sup>Breast Cancer Unit, François Baclesse Comprehensive Cancer Center; <sup>2</sup>Clinical Research Department, François Baclesse Comprehensive Cancer Center, Caen, Calvados, Normandy 14000, France

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**Abstract.** Cyclin-dependent-kinase 4-6 inhibitors (CDK4/6i) have improved the management of hormone receptor (HR)<sup>+</sup>/human epidermal growth factor receptor (HER)2<sup>-</sup> metastatic breast cancer (mBC). Currently, there are no valid prognostic factors for response to CDK4/6i. Baseline lymphopenia is reported as a prognostic factor in several types of cancer. The present retrospective study aimed to evaluate the effect of baseline absolute lymphocyte count (ALC) on response to palbociclib. Progression-free survival (PFS) was the primary endpoint. Secondary endpoints were overall survival (OS), best response and safety. A total of 114 patients treated for mBC between 2016 and 2019 were included. Median baseline ALC was 1.4 g/l (range, 0.2-4.3 g/l). A total of 65 (57%) and 49 (43%) patients had baseline ALC values of <1.5 and ≥1.5 g/l, respectively. Patients with baseline lymphopenia exhibited significantly shorter PFS (6 vs. 10 months; P=0.004) and OS (20 vs. 33 months; P=0.02). ALC <1.5 g/l independently predicted worse survival, as indicated by multivariate analysis (P=0.04; hazard ratio, 1.76; 95% confidence interval, 1.02-3.02). Patients with baseline ALC <1.5 g/l had significantly less partial response (14 vs. 22%; P=0.016) and more disease progression (46 vs. 20%; P=0.016) than those with ALC ≥1.5 g/l. ALC is a strong and easy-to-use dosage

with prognostic factor for patients with HR<sup>+</sup>/HER2<sup>-</sup> mBC treated with palbociclib and endocrine therapy. Lymphopenia may also be a predictive factor of early progression. These data need to be verified in a larger prospective study.

## Introduction

Breast cancer (BC) is the most frequently occurring cancer in women worldwide, with >2 million new cases diagnosed in 2018 (1). ~70% of BC cases are hormone receptor-positive (HR<sup>+</sup>) and human epidermal growth factor receptor 2-negative (HER2<sup>-</sup>). Endocrine therapy (ET) is considered the mainstay of treatment for both pre- and postmenopausal women with HR<sup>+</sup>/HER2<sup>-</sup> metastatic BC (mBC) (2). Regarding mBC, the most relevant therapeutic improvement of the last few years has been the introduction of cyclin-dependent kinase (CDK) 4 and 6 inhibitors (CDK4/6i) (palbociclib, ribociclib and abemaciclib) combined with ET. Pivotal trials, namely PALOMA 2 and 3, MONALEESA 2, 3 and 7, and MONARCH 2 and 3, showed an improvement in progression free survival (PFS) of 5 to 10 months (3-8). A recent meta-analysis reported that CDK4/6-i + ET combinations, compared with ET alone, improved overall survival (OS) independent of age, menopausal status, endocrine sensitivity and visceral involvement (9). Except for patients with extensive visceral involvement, CDK4/6i + ET combinations remain the treatment of choice for HR<sup>+</sup>/HER2<sup>-</sup> mBC (2).

Despite the significant improvements in survival determined by CDK4/6i, resistance represents a major clinical challenge. Resistance might be present immediately at treatment initiation. Primary or *de novo* resistance occurs in ~15% of patients receiving CDK4/6i with anti-aromatase inhibitors, and ~30% of those receiving CDK4/6i with fulvestrant (10). Currently, there are no valid prognostic factors for response to CDK4/6i. Baseline lymphopenia has been reported in several publications as a prognostic factor in different types of cancer (11-17). Thus, lymphopenia is an independent predictive factor of survival in metastatic colorectal cancer patient with shorter PFS (median 4 vs. 7 months; P=0.033) and OS (median 16 vs. 24 months, P=0.024)(11).

The present study aimed to assess the impact of baseline absolute lymphocyte count (ALC) on response to CDK4/6i.

**Correspondence to:** Dr George Emile, Breast Cancer Unit, François Baclesse Comprehensive Cancer Center, 3 Avenue du Général Harris, Caen, Calvados, Normandy 14000, France  
E-mail: g.emile@baclesse.unicancer.fr

**Abbreviations:** ALC, absolute lymphocyte count; BC, breast cancer; mBC, metastatic breast cancer; ET, endocrine therapy; CDK4/6i, cyclin dependent kinase 4-6 inhibitor; PFS, progression-free survival; OS, overall survival; AE, adverse event; HR<sup>+</sup>, hormone receptor-positive; HER2<sup>-</sup>, human epidermal growth factor receptor 2-negative; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; DCR, disease control rate; PS, performance status; HR, hazard ratio; TL, T lymphocytes

**Key words:** metastatic breast cancer, lymphopenia, cyclin dependant kinase inhibitor, palbociclib, prognosis factor

## Materials and methods

**Design.** Between April 2016 and February 2019, a descriptive retrospective single center study was performed at the François Baclesse Comprehensive Cancer Center, Caen, Calvados, Normandy. Eligible patients were women aged >18 years with HR<sup>+</sup>/HER2<sup>-</sup> mBC treated with palbociclib in combination with ET (an aromatase inhibitor or fulvestrant). Premenopausal women also received luteinizing hormone-releasing hormone agonists. A total of 114 patients were included; there were no predefined exclusion criteria. The primary end-point, PFS, was evaluated from palbociclib initiation to radiological progression, death or last follow-up. Secondary end points were OS (time from palbociclib initiation to death), best radiological response and safety. Tumor assessment was performed every 2-3 cycles and disease response was categorized as complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD), according to the response evaluation criteria in solid tumors (version 1.1) (18). Objective response rate (ORR) was defined as the percentage of patients in whom either CR or PR was observed. Disease control rate (DCR) was defined as the proportion of patients with either CR, PR or SD as best overall response. All patients underwent baseline routine blood tests, including white blood cell and ALC. Lymphopenia was defined as ALC <1.5 g/l; lymphopenia and other adverse events (AEs) were graded according to National Cancer Institute Common Terminology Criteria for AEs (version 5.0) (19). Initial dose of palbociclib and dose reductions were reported and analyzed for their impact on PFS and OS.

In accordance with regulations regarding research involving human subjects, the present study was registered in the corresponding data protection document. As an observational retrospective study, institutional review board approval was not required. Patients' non-opposition to the use of their data was sought after checking whether the patient was still living; as a result, no data exclusion due to death was necessary. All data were anonymized for statistical analysis.

**Statistical analysis.** Qualitative variables are presented as the number and frequency; quantitative variables are presented as the mean  $\pm$  standard deviation or median and extreme values. The characteristics of lymphopenic and non-lymphopenic patients were compared by  $\chi^2$  test (or Fisher's exact test, in case of observed values per category <5) for the qualitative variables, and by the unpaired Student's t-test for the quantitative variables (or Wilcoxon non-parametric test if data were not normally distributed).  $P < 0.05$  was considered to indicate a statistically significant difference. PFS and OS were calculated according to the Kaplan-Meier method and comparison of survival between different patient populations was performed by the log-rank or supremum log-rank test (Renyi-type test) in case of crossing curves. The impact of known prognostic factors (age, number of previous lines of treatment, palbociclib dose reduction and occurrence of AE) was assessed by univariate and multivariate Cox models. All incident cases were assessed (no calculation of the number of subjects needed). Analyses were conducted using R software, version 4.0.2 (<https://cran.r-project.org/bin/windows/base/>).

Table I. Characteristics of patients.

Characteristic	Number (n=114)	%
Median age, years (range)	51 (30-75)	
PS		
0	47	41.2
1	51	44.7
2	13	11.4
3	3	2.6
Histological diagnosis		
Invasive lobular carcinoma	21	19.6
Invasive ductal carcinoma	81	75.7
Other	5	4.7
Missing	7	6.1
Initial stage		
I-III	95	83.3
IV ( <i>de novo</i> )	19	16.7
Hormone receptor status		
ER <sup>+</sup> /PR <sup>+</sup>	94	82.5
ER <sup>+</sup> /PR <sup>-</sup>	19	16.6
ER <sup>-</sup> /PR <sup>+</sup>	1	0.9
Endocrine therapy		
Fulvestrant	97	85.1
Letrozole	17	14.9
ALC, g/l		
$\geq 1.50$	49	43.0
1.49-0.80	47	41.0
0.79-0.50	13	12.0
0.49-0.20	5	4.0

PS, performance status; ER, estrogen receptor; PR, progesterone receptor; ALC, absolute lymphocyte count.

## Results

**Clinicopathological data of patients.** Between April 2016 and February 2019, a total of 114 patients were recruited. The median age at palbociclib initiation was 51 years. Most patients had a good Eastern Cooperative Oncology Group performance status (PS; PS0, 41.2%; PS1, 44.7%). The median number of previous lines of treatment was four and 85.1% of patients received fulvestrant in combination with palbociclib. Only 16.7% of patients exhibited *de novo* mBC (Table I). Median baseline ALC was 1.4 g/l (range, 0.2-4.3 g/l). A total of 65 (57%) and 49 (43%) patients had baseline ALC <1.5 and  $\geq 1.5$  g/l, respectively. PS, number of previous lines of treatment and palbociclib dose reduction were not significantly different in these two groups (Table II).

**PFS was shorter in patients with lymphopenia.** Median PFS in the whole population was 7.9 months. Patients with baseline lymphopenia had significantly shorter PFS (6 vs. 10 months; log-rank  $P = 0.004$ ; Fig. 1). Univariate analysis demonstrated that age did not influence PFS. Patients who received

Table II. Characteristics of patients according to pretreatment absolute lymphocyte count.

Characteristic	Number		P-value
	ALC >1.5 g/l (n=49)	ALC <1.5 g/l (n=65)	
PS			0.170
0/1	45.0 (91.8%)	53.0 (81.5%)	
2/3	4.0 (8.2%)	12.0 (18.5%)	
Previous lines of treatment, median (range)	3.0 (2.0-4.0)	4.0 (2.0-6.0)	0.170
Best response			0.016 <sup>a</sup>
CR	1.0 (2.0%)	0.0 (0.0%)	
PR	11.0 (22.4%)	9.0 (13.8%)	
SD	27.0 (55.1%)	26.0 (40.0%)	
PD	10.0 (20.4%)	30.0 (46.2%)	
PFS, months (range)	10.0 (7.0-16.0)	6.0 (4.0-8.0)	0.004 <sup>a</sup>
OS, months (range)	33.0 (27.0-NA)	20.0 (17.0-27.0)	0.020 <sup>a</sup>
Adverse events			0.900
Grade 1/2	11.0 (26.2%)	14.0 (26.4%)	
Grade 3/4	31.0 (73.8%)	39.0 (73.6%)	

Adverse events are graded according to Common Terminology Criteria for Adverse Events V4.03. PS, performance status; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; PFS, progression-free survival; OS, overall survival; NA, not achieved. <sup>a</sup>P<0.05.

Table III. Univariate analysis of factors associated with progression-free survival.

Variable	HR	95% CI	P-value
Age <51 years	1.12	0.74-1.68	0.6000
Dose reduction of palbociclib	2.38	1.45-3.89	<0.0002 <sup>a</sup>
Baseline ALC <1.5 g/l	1.71	1.13-2.60	0.0115 <sup>a</sup>
Occurrence of adverse events	0.39	0.23-0.68	0.0088 <sup>a</sup>
<5 treatment lines	0.41	0.26-0.63	<0.0001 <sup>a</sup>

<sup>a</sup>P<0.05. ALC, absolute lymphocyte count.

Table IV. Multivariate analysis of factors associated with overall survival.

Variable	HR	95% CI	P-value
Age <51 years	0.79	0.48-1.30	0.3485
Dose reduction of palbociclib	1.42	0.80-2.51	0.2319
Baseline ALC <1.5 g/l	1.76	1.03-3.02	0.0399 <sup>a</sup>
Occurrence of adverse events	1.14	0.54-2.40	0.7245
<5 treatment lines	0.72	0.42-1.26	0.2517

<sup>a</sup>P<0.05. ALC, absolute lymphocyte count.

<5 previous lines of treatment had a significantly longer PFS (9 vs. 5 months; P<0.0001). Palbociclib dose reduction and the absence of AE were associated with worse PFS (6 vs. 8 months; P<0.0002 and 4 vs. 8 months; P=0.0088, respectively) (Table III). In multivariate analysis, age did not influence PFS. Lymphopenia and palbociclib dose reduction were associated with worse PFS [hazard ratio (HR)=1.71 (1.13-2.60); P=0.01 and HR=2.38 (1.45-3.89); P<0.001, respectively]. Presence of AE and <5 previous lines of treatment were significantly associated with better PFS [HR=0.39 (0.23-0.68) and 0.41 (0.26-0.64), respectively; P<0.001] (Table IV).

*OS was shorter in patients with lymphopenia.* Median OS in the whole population was 27 months. Patients with baseline lymphopenia had significantly shorter OS (20 vs. 33 months; log-rank P=0.018; supremum log-rank P=0.013; Fig. 1). In multivariate analysis, lymphopenia was independently

associated with worse OS [HR=1.76 (1.02-3.02); P=0.04]. Palbociclib dose reduction, occurrence of AE, age and number of lines of treatment did not have any impact on OS (Table III).

*Response rate was lower in patients with lymphopenia.* In the whole group, the ORR was 18.4% (21 patients), with CR achieved for one patient (1.7%). A total of 53 patients (46.5%) had SD. The PD rate was 35.1%, resulting in a DCR of 64.9% (74 patients). There was significantly less partial response (13.8 vs. 22.4%; P=0.016) and more disease progression at first disease evaluation (46.2 vs. 20.4%; P=0.016) in patients with baseline ALC<1.5 g/l compared with those with ALC≥1.5 g/l (Table II).

*Security data are compatible with those already known and reported in published phase 3 trials.* The majority of patients experienced hematological toxicity, as expected. A total of

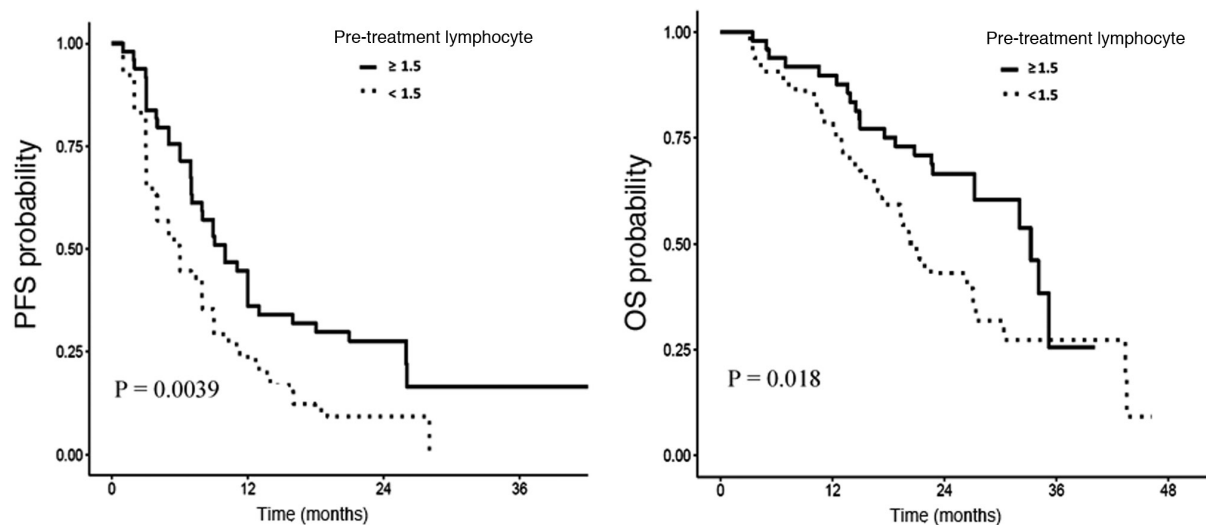


Figure 1. Kaplan-Meier plot comparing OS and PFS between patients with baseline lymphocytes  $\geq 1.5$  and  $< 1.5$  g/l. OS, overall survival; PFS, progression-free survival.

96 patients (84.2%) experienced AE. The most common AE was neutropenia (82.5%). More than 50% of patients had grade 3 or higher neutropenia and only one patient had febrile neutropenia. A total of eight patients (7%) experienced thrombocytopenia.

## Discussion

CDK4/6i and ET combinations are effective for most patients with HR<sup>+</sup>/HER2<sup>-</sup> mBC (9), but certain patients fail to respond and no biomarker is currently available to predict response to treatment (20). To the best of our knowledge, the present study is the first that demonstrates an association between baseline lymphopenia and worse survival and response rate in this population.

The host immune system serves a key role in cancer control (21). Lymphocytes, whether in peripheral blood or as tumor-infiltrating lymphocytes, are key factors contributing to the body's immune response (22-24). Baseline lymphopenia has been shown to be a poor prognostic factor for various types of cancer (11-17). For example, in a study by Ray-Coquard *et al* (17), lymphopenia was found to be an independent prognostic factor for OS and PFS in mBC [relative risk (RR), 1.8 ; 95% CI, 1.3-2.4], in advanced soft tissue sarcoma (RR, 1.46; 95% CI, 1.0-2.1) and in non-Hodgkin's lymphoma (RR, 1.48; 95% CI, 1.03-2.1). Lymphopenia is a powerful predictor of chemotherapy-induced toxicity and is also a predictive factor of the efficacy of chemotherapy in colorectal, breast and lung cancer (11,25-27).

Several studies have reported a worse ORR in patients with baseline lymphopenia compared with patients with normal ALC (11,25-27), as seen in the present study. Here, disease progression was observed at the first evaluation in 46.2% of patients with baseline lymphopenia vs. 20.4% with normal ALC (P=0.016). For HR<sup>+</sup>/HER2<sup>-</sup> BC, fewer data are available concerning the impact of lymphopenia on survival, although it is known that higher ALC is associated with better response to ET (28). Here, the majority of patients received fulvestrant (85%); to the best of our knowledge, the type of ET does not

influence ALC. More recently, it was reported that neutrophil-to-lymphocyte ratio is a predictive marker for response to ET in mBC (29). In the Ray-Coquard *et al* study (17) the ALC threshold was set at 1 g/l to predict OS in different types of tumor. In the present study, OS and PFS were impacted regardless of ALC.

Tumor cells can elude immune surveillance. One of the mechanisms of this escape is the recruitment of immunosuppressive regulatory T lymphocytes (TLs) (30). The previous success of immunotherapy based on anti-cytotoxic TL-associated antigen 4 or anti-programmed death-1/programmed death ligand-1 antibodies confirms the relevance of TL-based anti-tumor immunity and suggests that restoration of the immune system could promote tumor control (31). Several animal and *in vitro* models have demonstrated the immune actions of CDK4/6i (32-34). CDK4/6i increase the immunogenicity of tumor cells (32) and enhance tumor infiltration via TL activation (35,36). CDK4/6i enable reactivation of nuclear factor of activated T cell proteins and their target genes, including the gene encoding IL-2, a major cytokine that activates TL effectors (36). Finally, CDK4/6i may decrease the proliferation of regulatory TL (32,36), reversing the balance of TL effectors and TL regulators in favor of tumor control. CDK4/6i also increase expression of genes involved in antigen processing and presentation *in vivo* in mouse and patient-derived xenograft models and suppress the proliferation of immune-suppressive regulatory TL, thus promoting cytotoxic TL-mediated tumor cell destruction (35). Lymphopenia may reflect T cell dysfunction with limited ability to perform antitumor functions and immune actions during palbociclib therapy (37).

Both host characteristics and a high tumor burden can result in lymphopenia (38). In a pooled series, lymphopenia was associated with patient age (39). Inflammation-induced cell death and decreased thymic function have been suggested as potential mechanisms of peripheral lymphopenia observed in patients with metastasis (40). In our study, median age and PS were similar in patients regardless of ALC. A possible explanation is that our population was young (median age was only 51 years).



A large number of the patients in the present study were from the French Compassionate Access Program, which provides temporary authorization for use of unlicensed drugs outside of clinical trials to treat serious or rare diseases when no appropriate treatment exists. This program was implemented to improve early access to promising drugs (41). Thus patients were heavily pretreated, as in the Battisti *et al* study (42). This may explain the shorter PFS and OS in the present study compared with those reported in published registration studies (3-8). All patients received palbociclib because it was the first drug to have obtained marketing authorization in France. Lymphopenia was independent of the number of previous lines of treatment. Lymphopenia has been reported as a risk factor for the occurrence of chemotherapy-induced hematotoxicity, especially neutropenia, severe thrombocytopenia and anemia requiring transfusion and early death following chemotherapy (17,25,26,43,44). ALC of 0.7 g/l was previously identified as the most discriminative predictive value for hematological AE (45). Here, there was no association between baseline ALC and the probability of AE. Regarding dose reduction, the present results are consistent with previously published studies showing that reduce the dose of CDK4/6i has a negative impact on treatment efficacy (46,47).

A recent publication assessed the role of certain genomic markers in circulating tumor DNA to identify patients at higher risk of early progression following fulvestrant therapy in the presence or absence of palbociclib (48). The aforementioned study found that high-circulating tumor fraction, TP53 mutation and fibroblast growth factor receptor 1 amplification were associated with worse PFS even following the addition of CDK4/6i. Despite the interest in these genomic markers in prognostic estimation, they remain expensive and difficult to monitor in daily practice. The present study suggested that assessment of ALC, a routine and less expensive test, may serve as a significant prognostic factor for patients with HR<sup>+</sup>/HER2<sup>-</sup> mBC.

The present study had certain limitations, including the small sample size and retrospective single-center design. Due to lack of data, lactate dehydrogenase LDH dosage, an indicator of high tumor burden as suggested in another study (49), was not assessed. The present study did not have a long follow-up, however it was sufficient to obtain fairly discriminative survival information. Due to the limited sample size, retrospective design and heterogeneity of the population, other elements, such as markers of inflammation and CD4/8 TL count ratio could not be evaluated. The present results need to be confirmed by large-scale studies with extensive follow-up and assessment of other inflammation markers.

To the best of our knowledge, the present study is the first to demonstrate the impact of baseline lymphopenia as a strong and easy-to-use prognostic factor for patients with HR<sup>+</sup>/HER2<sup>-</sup> mBC treated with palbociclib in combination with ET. Lymphopenia may also be a predictive factor of early progression. A larger study is needed to confirm these results.

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## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Authors' contributions

GE was responsible for the conception and design of the study. GE, SP and ADS collected the data and wrote the manuscript. GE and ADS confirm the authenticity of all the raw data. JL performed statistical analysis, participated in data analysis, interpreted the data and wrote the manuscript. GE, SP, ADS, CL, AJ, DA, IH, CS, AM, KG, AF and FC interpreted the data and wrote the manuscript. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

In accordance with the regulations regarding research involving human subjects, the present study was registered with corresponding data protection. Patients' non-opposition to the use of their data was sought after verification of their vital status.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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