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ORIGINAL PAPER

Prognostic value of CEC count in HER2-negative metastatic breast cancer patients treated with bevacizumab and chemotherapy: a prospective validation study (UCBG COMET)

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Abstract

Background Proof of concept studies has reported that circulating endothelial cell (CEC) count may be associated with the outcome of HER2-negative metastatic breast cancer (mBC) patients treated by chemotherapy and the anti-VEGF antibody bevacizumab. We report the results obtained in an independent prospective validation cohort (COMET study, NCT01745757).

Methods The main baseline criteria were HER2-negative mBC, performance status 0–2 and no prior chemotherapy for metastatic disease. CECs were detected by CellSearch® from 4 ml of blood at baseline and after 4 weeks of weekly paclitaxel and bevacizumab therapy. CEC counts (considered both as a continuous variable and using the previously described 20 CEC/4 ml cutoff) were associated with clinical characteristics and progression-free survival (PFS).

Results CEC count was obtained in 251 patients at baseline and in 207 patients at 4 weeks. Median baseline CEC count was 22 CEC/4 ml (range 0–2231). Baseline CEC counts were associated with performance status ($p = 0.02$). No statistically significant change in CEC counts was observed between baseline and 4 weeks of therapy. High baseline CEC count was associated with shorter PFS in univariate and multivariate analyses (continuous: $p < 0.001$; dichotomized: HR 1.52, 95% CI [1.15–2.02], $p = 0.004$). CEC counts at 4 weeks had no prognostic impact.

Conclusion This study confirms that CEC count may be associated with the outcome of mBC patients treated with chemotherapy and bevacizumab. However, discrepancies with previous reports in terms of both the timing of CEC count and the direction of the prognostic impact warrant further clinical investigation.

Keywords Bevacizumab · Breast cancer · Circulating endothelial cells

Introduction

It has been suggested that standard chemotherapy (including taxanes) has a stimulant effect on endothelial progenitors [1], which may explain why combinations of paclitaxel and antiangiogenic agents have demonstrated prolonged progression-free survival (PFS) in HER2-negative mBC [2–4]. Nevertheless, the addition of bevacizumab did not increase overall survival (OS) of mBC patients [5]. Due to the increased toxicity and the lack of OS benefit in the overall HER2-negative mBC population, some pharmaceutical regulatory agencies (e.g., the US Food and Drug Administration) subsequently withdrew the mBC indication of bevacizumab, while others (e.g., the European Medicines Agency (EMA)) restricted the use of bevacizumab to triple-negative mBC.

Various biomarkers have been studied in order to predict the response to bevacizumab, particularly circulating endothelial cells (CECs). These cells are considered to be a biomarker of vascular damage and dysfunction and have been reported to be increased in patients with breast cancer [6]. The recurring problem in CEC monitoring is the lack of a standardized method for their detection. Various

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techniques have been used in previous studies, such as flow cytometry [7] or the semi-automated CellSearch® system, validated for circulating tumor cell detection [8]. Bevacizumab has been demonstrated to be associated with variations in CEC counts [9, 10] in many cancer types, which could make CECs an interesting biomarker for monitoring the response to bevacizumab. Using flow cytometry, Calleri et al. [7] showed that CEC counts in mBC increased during bevacizumab therapy and decreased at the time of tumor progression. Similar observations were also reported by our team in metastatic [11] and early [12] breast cancer patients, using the CellSearch® system. More specifically, in 67 mBC patients treated with first-line chemotherapy and bevacizumab, we reported that patients with ≥ 20 CEC/4 ml after 4 weeks on treatment had a significantly longer time to progression [11].

This study was designed to validate the prognostic role of CEC count in mBC patients treated with first-line bevacizumab and chemotherapy.

Materials and methods

This article has been written in accordance with the REporting of tumor MARKer studies criteria [13].

Patients and treatment

COMET is a prospective, multicenter, single-arm cohort study focusing on bevacizumab-related biomarkers. Participation in the study was proposed to mBC patients about to receive first-line therapy with weekly paclitaxel and bevacizumab. As per the 2012 EMA label for bevacizumab, patients could have triple-negative or estrogen receptor-positive (ER +), HER2-negative breast cancer. Other baseline criteria were: age > 18 years, performance status (PS) of 0–2, life expectancy ≥ 12 weeks and written informed consent. Exclusion criteria were prior chemotherapy for mBC, concomitant endocrine therapy or radiation therapy with curative intent for oligometastatic disease.

All patients received intravenous paclitaxel 90 mg/m^2 on days 1, 8 and 15 with bevacizumab 10 mg/kg on days 1 and 15. Treatment was repeated every 4 weeks, according to routine practice, until disease progression or unacceptable toxicity. The study was approved by an ethics committee (*Comité de Protection des Personnes "Ile de France VII"*) in June 2012 and registered (NCT01745757).

CEC detection

Blood was drawn in CellSave® tubes at baseline and at 4 weeks, maintained at room temperature and processed in a central laboratory at Institut Curie (Paris, France) within 72 h. The standardized CellSearch® technique for CEC [14] detection has been reported previously. Briefly, CECs expressing CD146 were immuno-magnetically enriched and stained with DAPI (+), while CD105 (+) and CD45 (−) status was assessed by immunocytofluorescence. The complete description of the CEC detection methods is described elsewhere and provided a gallery of typical CEC images [14]. The image software used for this analysis identifies CEC as cells that are CD146 (+), CD105 (+) and CD45(−); a trained technician confirmed whether the object meets all CEC morphology criteria [14]. Quantitative results are expressed per 4 ml blood. All evaluations were carried out by qualified technicians with no knowledge of the patient's clinical status.

Statistical analysis

Based on our prior study [11], the number of subjects to be included was calculated in order to detect a hazard ratio of 1.6 for death or progression in patients with CEC levels at 4 weeks < 20 CEC/4 ml as compared to patients with CEC levels ≥ 20 CEC/4 ml. We estimated that patients would be equally distributed between the two groups. Assuming a 6-month PFS of 75%, an enrollment period of approximately 18 months, a minimum follow-up of 2 years, 185 patients were required to provide the study a power of 80% with a two-sided log-rank test at a significance level of 5%. Taking into account 10% of missing CEC data at 4 weeks, at least 206 patients had to be included.

Associations between patient characteristics and dichotomized CEC levels were studied with Chi-square tests. When treated as continuous, associations with CEC were tested

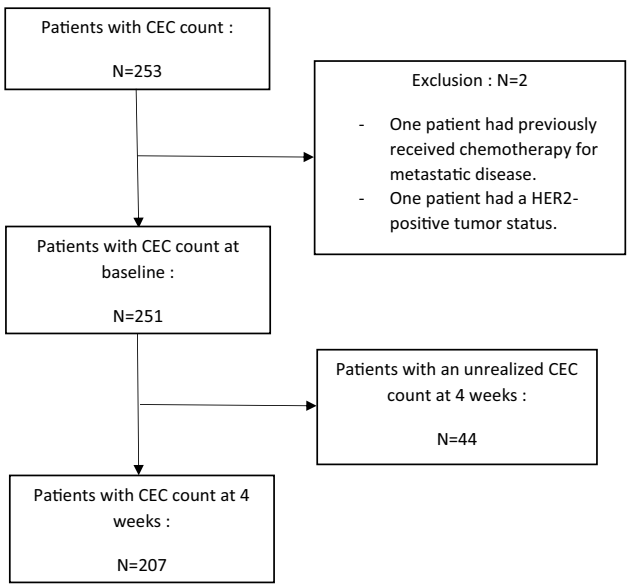

Fig. 1 Flowchart

Table 1 Patient characteristics and association between CEC count at baseline and patient characteristics

Characteristics	Number of patients (%)	Baseline CEC count			
		Dichotomized			Continuous
		< 20 CEC/4 ml *N* (%)	≥ 20 CEC/4 ml *N* (%)	*P* value	*P* value
Sex					
Female	250 (99.6%)	112 (99%)	138 (100%)	0.9	0.8
Male	1 (0.4%)	1 (1%)	0		
Age					
< 50 years	77 (31%)	35 (31%)	42 (30%)	0.9	0.7
≥ 50 years	174 (69%)	78 (69%)	96 (70%)		
Menopausal status					
Premenopausal	68 (27%)	33 (30%)	35 (26%)	0.6	0.6
Postmenopausal	178 (73%)	76 (70%)	102 (74%)		
Missing data	5	4	1		
Performance status					
0	141 (56%)	73 (64%)	68 (49%)	0.02	0.005
1	98 (39%)	38 (34%)	60 (44%)		
2	12 (5%)	2 (2%)	10 (7%)		
SBR grade					
1	19 (8%)	9 (8%)	10 (8%)	0.4	0.4
2	114 (50%)	47 (45%)	67 (54%)		
3	96 (42%)	49 (47%)	47 (38%)		
Missing data	22	8	14		
Subtype					
Triple-negative	51 (22%)	22 (21%)	29 (23%)	0.9	0.3
Hormone receptor-positive	182 (78%)	83 (79%)	99 (77%)		
Missing data	18	8	10		
Metastasis-free interval					
0 months	27 (11%)	14 (13%)	13 (10%)	0.6	0.9
[0–24] months	57 (23%)	23 (20%)	34 (25%)		
> 24 months	162 (66%)	75 (67%)	87 (65%)		
Missing data	5	1	4		
Number of metastatic sites					
< 3	223 (90%)	105 (95%)	118 (87%)	0.06	0.06
≥ 3	24 (10%)	6 (5%)	18 (13%)		
Missing data	4	2	2		
Visceral sites					
Yes	185 (77%)	80 (75%)	105 (79%)	0.5	0.4
No	55 (23%)	27 (25%)	28 (21%)		
Missing data	11	6	5		
Prior (neo)adjuvant chemotherapy					
Yes	171 (68%)	74 (65%)	97 (70%)	0.5	0.6
No	80 (32%)	39 (35%)	41 (30%)		
Prior endocrine therapy					
Yes	148 (59%)	64 (57%)	84 (61%)	0.6	0.7
No	103 (41%)	49 (43%)	54 (39%)		
Total	251	113	138		

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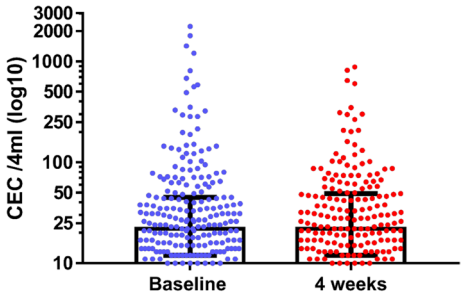

Fig. 2 Distribution of CEC value at baseline and at 4 weeks

with Mann–Whitney or Kruskal–Wallis tests. Distributions of CEC count between baseline and 4 weeks were compared with the Wilcoxon sign-rank test for paired data.

PFS was defined as the time to disease progression or death of any cause. It was measured from the inclusion or the date of second sampling (4 weeks) for the analyses based on CEC levels at 4 weeks. PFS curves were computed by the Kaplan–Meier method. Univariate analyses of PFS according to dichotomized CEC levels and other potential prognostic factors (Table 3) were performed with the use of a two-sided log-rank test. Variables significant at the 0.10 level in univariate analyses (Table 3) were entered in a multivariable Cox proportional hazards model with backward stepwise baseline of factors. Due to the well-known problems introduced by categorization of quantitative variables, and in order not to miss a significant effect of CEC, a multivariable analysis was also performed with a multiple fractional polynomial (MFP) Cox model to investigate possible nonlinear functional relationships between PFS and CEC treated as continuous. The same methodology (MFP Cox model) was used to assess the prognostic role of the variation in CEC levels between baseline and 4 weeks on PFS.

Results

Patients

Between September 2012 and September 2014, CEC counts were determined for 253 patients from 14 centers. Two patients were excluded from the study as they failed to meet the study baseline criteria: One patient had previously received chemotherapy for metastatic disease and the other patient's tumor status was ultimately evaluated as HER2-positive. Flowchart is shown (Fig. 1); the second time point, a drop-out rate of 18% ($n=44$ samples missing), was observed, in keeping with other observational multicenter studies on circulating tumor biomarkers [15–17]. Clinicopathological characteristics of the 251 patients included in this analysis are given in Table 1.

The median age of these patients was 58 years (IQR [47–65]). Fifty-one (21.9%) patients had triple-negative breast cancer. Most patients had received prior neoadjuvant/adjuvant chemotherapy ($n=171$, 68.1%) or endocrine therapy ($n=148$, 59%). Patients presented a small number of metastatic sites ($n=227$, 90.3% had less than three metastatic sites), most of which were visceral sites ($n=185$, 77.1%). One hundred sixty-two (65.5%) patients had a metastasis-free interval greater than 24 months, while 49 (19.9%) patients had a synchronous de novo mBC.

Median follow-up in this cohort was 58 months. The median number of treatment cycles was 8. Two hundred thirty-five PFS events were observed, and the median PFS was 10.0 months (95% CI [8.9–11.4]).

CEC detection

The median CEC count among the 251 patients with an available baseline CEC count was 22 CEC/4 ml (range: 0–2231, IQR [12–45]); 113 (45%) and 138 (55%) patients had CEC counts <20 and ≥ 20 CEC/4 ml, respectively. Median CEC count was very similar (22 CEC/4 ml) at 4 weeks (range: 1–881, IQR [12–48.5]) in the 207 patients assessed at both time points ($p=0.9$, Fig. 2). Among the 207 patients with both baseline and 4-week CEC counts available, 54 (26%), 35 (17%), 39 (19%), 79 (38%) had <20 CEC/4 ml at both baseline and 4 weeks, <20 CEC/4 ml at baseline then ≥ 20 CEC/4 ml at 4 weeks, ≥ 20 CEC/4 ml at baseline then <20 CEC/4 ml at 4 weeks and ≥ 20 CEC/4 ml at both baseline and 4 weeks, respectively.

Association between CEC count and patient characteristics and outcome

Associations between baseline CEC ($<20, \geq 20$) count and patient characteristics are given in Table 1: CEC count was significantly associated with performance status (PS) ($p=0.02$), while a non-significant association was observed with the number of metastatic sites ($p=0.06$). Similar results were observed at 4 weeks with CEC expressed as a dichotomized variable ($p=0.005$) (Table 2). At baseline, CEC count expressed as a continuous variable was significantly associated with PS ($p=0.005$) and at 4 weeks with PS ($p=0.002$) and visceral sites metastasis ($p=0.04$).

Baseline CEC count had a prognostic impact in univariate analysis when dichotomized using the cut-off of ≥ 20 CEC/4 ml (Fig. 3A, log rank $p=0.006$), as patients with ≥ 20 CEC/4 ml experienced a shorter PFS (median PFS: 8.6 months, 95% CI [7.2–10.2]) than those with <20 CEC/4 ml (median PFS: 12.0 months, 95% CI [10.4–14.7]). Other factors associated with shorter PFS in univariate analysis were: triple-negative status ($p<0.0001$), grade 3 tumors ($p=0.003$), ≥ 3 metastatic sites ($p=0.07$) and

Table 2 Patient characteristics and association between CEC count at 4 weeks and patient characteristics

Characteristics	Number of patients (%)	4-week CEC count			
		Dichotomized			Continuous
		< 20 CEC/4 ml *N* (%)	≥ 20 CEC/4 ml *N* (%)	*P* value	*P* value
Sex					
Female	206 (99.5%)	89 (100%)	117 (99%)	1	0.9
Male	1 (0.5%)	0	1 (1%)		
Age					
< 50 years	65 (31%)	31 (35%)	34 (29%)	0.4	0.5
≥ 50 years	142 (69%)	58 (65%)	84 (71%)		
Menopausal status					
Premenopausal	57 (28%)	28 (33%)	29 (25%)	0.3	0.5
Postmenopausal	146 (72%)	58 (67%)	88 (75%)		
Missing data	4	3	1		
Performance status					
0	122 (59%)	63 (71%)	59 (50%)	0.005	0.002
1	76 (37%)	25 (28%)	51 (43%)		
2	9 (4%)	1 (1%)	8 (7%)		
SBR grade					
1	15 (8%)	7 (9%)	8 (7%)	0.6	0.7
2	96 (51%)	43 (54%)	53 (49%)		
3	76 (41%)	29 (37%)	47 (44%)		
Missing data	20	10	10		
Subtype					
Triple-negative	38 (20%)	17 (20%)	21 (19%)	1	0.9
Hormone receptor-positive	153 (80%)	66 (80%)	87 (81%)		
Missing data	16	6	10		
Metastasis-free interval					
0 months	22 (11%)	8 (9%)	14 (12%)	0.7	0.8
[0–24] months	44 (22%)	21 (24%)	23 (20%)		
> 24 months	136 (67%)	58 (67%)	78 (68%)		
Missing data	5	2	3		
Number of metastatic sites					
< 3	185 (90%)	82 (92%)	103 (88%)	0.5	0.4
≥ 3	21 (10%)	7 (8%)	14 (12%)		
Missing data	1	0	1		
Visceral sites					
Yes	155 (77%)	61 (71%)	94 (82%)	0.1	0.04
No	46 (23%)	25 (29%)	21 (18%)		
Missing data	6	3	3		
Prior (neo)adjuvant chemotherapy					
Yes	141 (68%)	63 (71%)	78 (66%)	0.6	0.2
No	66 (32%)	26 (29%)	40 (34%)		
Prior endocrine therapy					
Yes	126 (61%)	53 (60%)	73 (62%)	0.8	0.9
No	81 (39%)	36 (40%)	45 (38%)		
Total	207	89	118		

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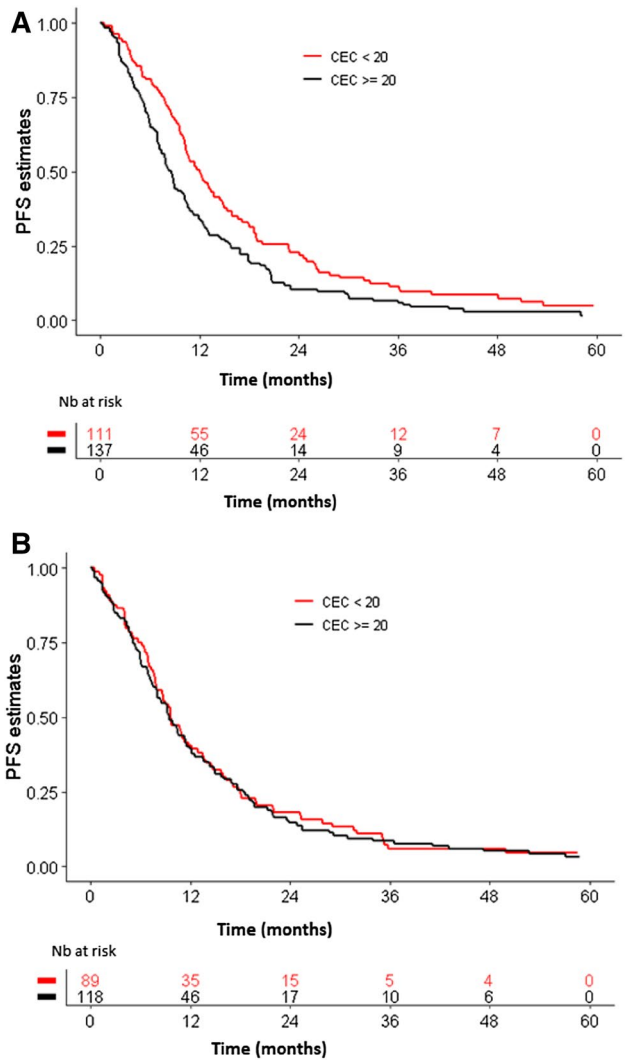

Fig. 3 PFS according to baseline (**A**) and 4-week (**B**) CEC counts. **A** Baseline CEC < 20/4 ml (red curve): median PFS = 12 months, 95% CI [10.4–14.7], CEC ≥ 20 CEC/4 ml (black curve): median PFS = 8.6 months, 95% CI [7.2–10.2], $p = 0.006$. **B** Four-week CEC < 20/4 ml (red curve): median PFS = 9.6 months, 95% CI [7.8–12.9], CEC ≥ 20 CEC/4 ml (black curve): median PFS = 9.4 months, 95% CI [8.0–11.9], $p = 0.7$

prior endocrine therapy ($p = 0.03$) (Table 3). In multivariate analysis, three significant variables remained predictive of poor prognosis at baseline: triple-negative status (HR 2.14, 95% CI [1.51–3.04], $p < 0.0001$), grade 3 (HR 1.57, 95% CI [1.18–2.1], $p < 0.002$) and baseline CEC count ≥ 20/4 ml (HR 1.52; 95% CI [1.18–2.02], $p = 0.004$) (Table 4).

CEC count at 4 weeks did not have a prognostic impact in univariate analysis using the cutoff of ≥ 20 CEC/4 ml (Fig. 3B, log rank $p = 0.7$): Patients with ≥ 20 CEC/4 ml have a similar PFS (median PFS: 9.4 months, 95% CI [8.0–11.9]) compared with the patients with < 20 CEC/4 ml (median PFS: 9.6 months, 95% CI [7.8–12.9]). Prognostic factors for PFS at 4 weeks were similar to those at baseline (Tables 3, 4).

Moreover, changes in CEC count during therapy according to baseline and 4-week counts were not significantly associated with PFS (Fig. 4). Among the 44 patients who did not have CEC dosage at 4 weeks, median PFS (10.5 months, 95% CI [9.6–12.3]) was similar than patients at baseline (10.0 months, 95% CI [8.9–11.4]).

Discussion

To the best of our knowledge, this is the largest prospective study of CEC count in mBC patients treated with the antiangiogenic agent bevacizumab combined with chemotherapy. We report that higher baseline CEC counts are an independent prognostic factor, together with triple-negative status and high tumor grade.

CECs are difficult to detect because of the small number of cells present in blood, between 0.01 and 0.0001% of all mononuclear cells [18]. CECs corresponding to those cells desquamated from vascular luminal endothelium, which is a dynamic, perpetually remodeling structure, resulting in various CEC phenotypes, which consequently impacts their detection. CECs are usually quantified by two different techniques: flow cytometry or immunomagnetic detection system [19]. While flow cytometry is able to characterize different CEC subpopulations, including CEC progenitors, its workflow requires immediate handling of the patient's blood, whereas immunomagnetic detection allows centralized testing with a more clinically friendly timing. Cells sorted by the CellSearch® based on their CD146(+), CD105(+), DAPI(+) and CD45(−) phenotype had their endothelial origin validated by gene expression profiling such as vascular endothelial cadherin [20]. Comparative studies have also shown a good overall concordance between CellSearch® (or other immunomagnetic bead-based assay) and flow cytometry, although cytometry appeared to be more sensitive [21, 22]; the CellSearch® system was also being used as a benchmark for new cytometry panels [23]. In our study, CellSearch® technology was chosen for its high reproducibility and because samples needed to be transported for centralized analysis. The recognition technique used for CEC detection (CD146 (+), DAPI (+), while CD105 (+) and CD45 (−)) is debated, and many pre-clinical or clinical studies used CD34 + and/or CD31 + (or other antigens) for the detection of CEC [24]. Therefore, using a different method of CEC detection could provide different results in terms of CEC detection and counting.

The number of CEC detected with the CellSearch® system in the ongoing COMET study is in keeping with our previous report on 67 mBC patients, in which the median CEC count was 17 CEC/4 ml (range: 1–769) [11]. A smaller

Table 3 Univariate analysis of prognostic factors measured at baseline and at 4 weeks

Characteristics	At baseline		At 4 weeks	
	Median PFS (months) 95% CI	*P* value	Median PFS (months) 95% CI	*P* value
CEC				
< 20 CEC/4 ml	12 [10.4–14.7]	0.006	9.6 [7.8–12.9]	0.7
≥ 20 CEC/4 ml	8.6 [7.2–10.2]		9.4 [8.0–11.9]	
Age				
< 50	9.9 [8.2–12.4]	0.9	9.1 [7.8–13.5]	0.7
≥ 50	10.2 [8.8–11.9]		9.6 [8.5–11.7]	
Performance status				
0	10.2 [8.9–12.4]	0.6	9.7 [8.7–12.1]	0.5
1–2	9.9 [8.3–11.9]		9.4 [7.6–117]	
Subtype				
Hormone receptor-positive	12.2 [10.6–13.8]	< 0.0001	11.5 [10.3–14.2]	< 0.0001
Triple-negative	5.5 [4.2–7.2]		4.8 [3.2–6.5]	
SBR grade				
1–2	12.3 [10.5–15.3]	0.003	12.9 [10.3–16]	0.008
3	8.7 [7.8–10]		7.8 [6.9–9.3]	
Metastasis-free interval				
0 month	9.4 [5.9–15.9]	0.6	9.9 [7.1–17.7]	0.7
[0–24] months	7.1 [6.4–8.9]		6.5 [5.0–9.6]	
> 24 months	10.8 [10.2–12.9]		10.8 [9.4–13.4]	
Number of metastatic sites				
< 3	10.2 [8.9–12]	0.07	9.9 [9.1–11.9]	0.01
≥ 3	8.9 [7.2–13.1]		7.7 [5.5–12.2]	
Visceral sites				
No	12.6 [8.9–16.9]	0.3	13.4 [9.7–18.1]	0.2
Yes	9.6 [8.5–10.7]		9.3 [7.6–10.4]	
Prior (neo)adjuvant chemotherapy				
Yes	8.9 [7.8–10.8]	0.17	9.1 [7.5–11.3]	0.4
No	10.7 [9.6–13.4]		10.4 [9.3–13.6]	
Prior endocrine therapy				
Yes	10.8 [10.1–12.6]	0.03	10.6 [9.4–12.9]	0.9
No	8 [6.9–10.6]		7.3 [6–10.7]	

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Table 4 Multivariate analysis of prognostic factors at baseline

Adverse prognostic factors	Hazard ratio [95% CI]	*P* value
Triple-negative	2.14 [1.51–3.04]	< 0.001
SBR grade 3	1.58 [1.18–2.10]	0.002
Baseline CEC ≥ 20/4 ml	1.52 [1.15–2.02]	0.004

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study, conducted in 23 mBC patients, reported a median CEC count of 122 CEC/4 ml with the same technique [25]. We report an association between CEC count and PS at inclusion. This association was described in non-small-cell lung cancer [26, 27] and colorectal cancer [28]. We hypothesize that both CEC count and PS might be related to the metastatic tumor burden, which was not assessed in our study.

Regarding the outcome of mBC patients treated with chemotherapy and bevacizumab, the present study was designed to validate our previous report suggesting that an increase in CEC count ≥ 20 CEC/4 ml after 4 weeks on treatment was associated with a longer time to progression [11]. Despite the use of the same CellSearch® technique, our results differ from those of the previous study: In this study, we report that a high baseline CEC count ≥ 20 CEC/4 ml was an independent adverse prognostic factor, but that CEC changes and the 4-week CEC count had no significant prognostic impact. These discordant results cannot be fully explained, as patient populations were very similar

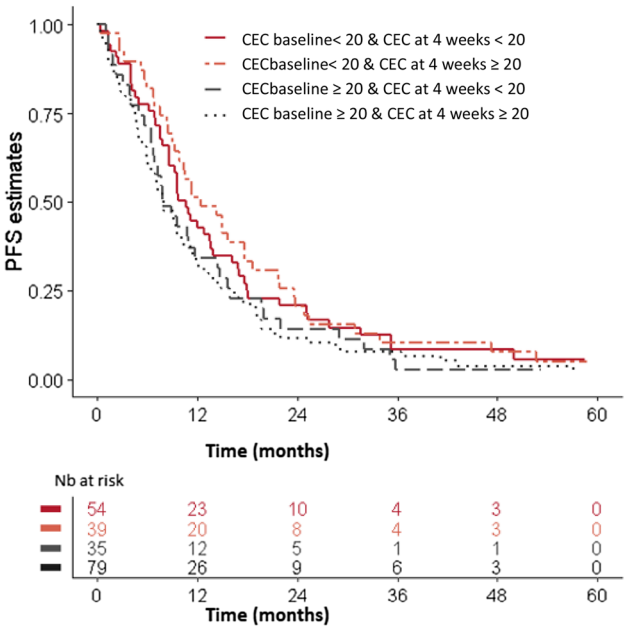

Fig. 4 PFS according to changes in CEC count during treatment. Changes in CEC count during treatment, when treated as continuous, were not significantly associated with PFS. Patients with ≥ 20 CEC/4 ml at baseline and < 20 CEC/4 ml at 4 weeks had a similar PFS (median PFS: 7.8 months) to patients with ≥ 20 CEC/4 ml at 4 weeks (median PFS: 8.0 months). Patients with < 20 CEC/4 ml at baseline and < 20 CEC/4 ml at 4 weeks have a median PFS of 10.7 months, while patients with < 20 CEC/4 ml at baseline and ≥ 20 CEC/4 ml at 4 weeks have a median PFS of 12.5 months

(HER2-negative mBC patients treated with first-line therapy) and received the same bevacizumab doses and chemotherapy backbone. In another study, Calleri et al. reported that high baseline CEC count was significantly associated with longer PFS in mBC patients treated with chemotherapy and bevacizumab as first or later lines of treatment. However, this result was obtained with a different CEC detection technique (flow cytometry) and in an overall small number of patients ($n = 46$) [7]. Similar contradictory results have also been reported in non-metastatic inflammatory (T4dNxM0) breast cancer patients treated by neoadjuvant chemotherapy and bevacizumab: In the Beverly 01 and 02 trials, higher pathological complete response rates were observed in patients with ≥ 20 CEC/4 ml during neoadjuvant therapy and < 20 CEC/4 ml at completion of neoadjuvant therapy, respectively [29, 30]. The design of our single-arm clinical study however prevents us to drawn definitive conclusions about the respective contributions of bevacizumab and chemotherapy to our results. It has to be noted that, besides bevacizumab, weekly paclitaxel could be considered as a metronomic drug and exerts some antiangiogenic activity [31, 32]. Interestingly, a small study in metastatic colorectal cancer patients suggested that the prognostic impact of baseline CEC count was observed only in bevacizumab-treated patients and not in patients receiving chemotherapy without bevacizumab [33].

While the use of chemotherapy and bevacizumab is mostly discontinued in mBC patients, bevacizumab is currently investigated as a potential enhancer of the efficacy of immunotherapy in triple-negative mBC (NCT03424005).If such an approach proves to be successful, the independent prognostic value of the baseline CEC count, as observed in our study and pending further validation, might be proposed as a stratification biomarker and/or selection criterion.

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Compliance with ethical standards

Conflict of interest JY Pierga received lecture honoraria, travel grant and research funding from Roche; FC Bidard received travel grant and research funding from Roche, Menarini Silicon Biosystems; A Goncalves received travel, accommodation and meeting registration support from Pfizer, Novartis, Roche, AstraZeneca, MSD, Celgene; O Tredan received honoraria from Roche, Novartis, AstraZeneca, Pfizer, Lilly and MSD for boards and symposiums. Other authors have stated explicitly that they have no conflicts of interest in connection with this article.

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