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ORIGINAL REPORT

Metronomic Cyclophosphamide and Capecitabine Combined With Bevacizumab in Advanced Breast Cancer

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ABSTRACT

Purpose
Metronomic chemotherapy has shown efficacy in patients with metastatic breast cancer. When used in association with targeted antiangiogenic drugs, it was more active than metronomic therapy alone in preclinical and clinical studies.

Patients and Methods
Patients with advanced breast cancer were candidates to receive metronomic oral capecitabine (500 mg thrice daily) and cyclophosphamide (50 mg daily) plus bevacizumab (10 mg/kg every 2 weeks).

Results
In 46 assessable patients, we observed one complete response (CR; 2%), 21 partial responses (PR; 46%), 19 patients (41%) with stable disease (SD), and five patients (11%) with progressive disease, for an overall response rate of 48% (95% CI, 33% to 63%). Additional long-term disease stabilization (SD ≥ 24 weeks) occurred in eight patients, for an overall clinical benefit (CR + PR + SD ≥ 24 weeks) of 68% (95% CI, 51% to 81%). Median time to progression was 42 weeks (95% CI, 26 to 72 weeks). Toxicity was generally mild. Grade 3 or 4 nonhematologic adverse effects included hypertension (n = 8), transaminitis (n = 2), and nausea/vomiting (n = 2). Higher baseline circulating endothelial cells (CECs) were correlated with overall response ($P = .02$), clinical benefit ($P = .01$), and improved progression-free survival ($P = .04$).

Conclusion
Treatment with metronomic capecitabine and cyclophosphamide in combination with bevacizumab was effective in advanced breast cancer and was minimally toxic. The number of baseline CECs significantly correlated with response and outcome, therefore supporting further studies on this surrogate marker for the selection of patients to be candidates for antiangiogenic treatments.

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INTRODUCTION

Metronomic chemotherapy refers to the frequent, even daily, administration of chemotherapeutics at doses significantly less than the maximum-tolerated dose, with no prolonged drug-free breaks.[1] An antiangiogenic activity is prominent with the protracted exposure to low doses of chemotherapeutics, if compared with their cyclic administration at the maximum-tolerated dose.[2] We previously showed that the administration of oral cyclophosphamide 50 mg daily and oral methotrexate 2.5 mg twice daily 2 days per week induced a response rate (RR) of 19% and a 32% rate of clinical benefit (CB) in the absence of serious toxicity and with a marked decrease in circulating vascular endothelial growth factor (VEGF).[3,4]

Bevacizumab is a recombinant humanized monoclonal immunoglobulin G1 antibody against human VEGF. It binds to VEGF, preventing its interaction with the VEGF receptor tyrosine kinases VEGFR1 and VEGFR2. Preclinical studies clearly show that bevacizumab reduces tumor angiogenesis and inhibits the growth of solid tumors.[5]

There is a rationale and evidence for the combination of metronomic chemotherapy and targeted antiangiogenic agents like bevacizumab. In preclinical models, the combination of metronomic chemotherapy with a VEGFR2 antibody resulted in sustained regressions of large tumors, without overt toxicity occurring during the course of treatment.[6] Moreover, the preclinical combination of metronomic cyclophosphamide, bevacizumab, and trastuzumab was more effective than cyclophosphamide and trastuzumab in

delaying tumor growth.[7] A randomized phase II trial comparing metronomic cyclophosphamide and methotrexate with the same regimen plus bevacizumab in women with pretreated advanced breast cancer was recently presented.[8] A planned interim analysis after the first 19 patients per arm revealed a significant advantage in favor of the combined arm in terms of objective remissions (41%). Similarly, a combination of daily low-dose cyclophosphamide and bevacizumab was shown to induce encouraging activity in a nonrandomized phase II trial of 70 patients with recurrent ovarian cancer.[9]

Capecitabine has proven activity in advanced breast cancer. Retrospective analyses demonstrated that lower doses have a more favorable therapeutic index in metastatic breast cancer (MBC) when compared with standard dosage.[10] Moreover, fixed daily doses and continuous (noncyclic) dosing schedules have been demonstrated to be well tolerated and active in breast cancer.[11] In a phase III trial, the combination of capecitabine and bevacizumab was more active in terms of objective remissions when compared with capecitabine alone (19.8% *v* 9.1%, respectively; $P = .001$).[12] A synergistic effect was observed with the metronomic combination of a fluorouracil prodrug and cyclophosphamide. In a recent preclinical study involving treatment of human advanced systemic MBC in immune-deficient mice, the combination of long-term daily low-dose cyclophosphamide and uracil-ftorafur induced long-term survival with minimal adverse effects.[13]

Given these considerations, we designed a phase II trial to explore the activity and tolerability of a regimen combining metronomic cyclophosphamide (50 mg daily) plus metronomic capecitabine (500 mg thrice daily) with bevacizumab (10 mg/kg intravenous every 2 weeks). Considering the virtual absence of factors able to predict response to targeted antiangiogenic treatments, we explored the correlation of circulating endothelial cells (CECs) and circulating endothelial progenitors (CEPs) with the response and outcome of the patients.

PATIENTS AND METHODS

The trial was conducted at the European Institute of Oncology, Milan, Italy. Patients age 18 to 80 years with histologically proven breast cancer with the following characteristics were eligible: pre- or postmenopausal, locally advanced (inoperable) or metastatic breast carcinoma; measurable disease; no more than three previous lines of chemotherapy for advanced disease (primary and/or adjuvant chemotherapy was allowed, as was any prior endocrine treatment); at least 4 weeks must have elapsed since prior chemotherapy or radiation therapy (6 weeks if the last regimen included mitomycin); life expectancy greater than 6 months; Eastern Cooperative Oncology Group performance status less than 2 (Karnofsky performance status $> 60\%$); normal organ and marrow function (leukocytes $\geq 3{,}000/\mu L$, absolute neutrophil count $\geq 1{,}500/\mu L$, platelets $\geq 100{,}000/\mu L$, total bilirubin within normal institutional limits, AST/ALT $\leq 2\times$ institutional upper limit of normal, creatinine within normal institutional limits, or creatinine clearance ≥ 60 mL/min); absence of cerebral or leptomeningeal involvement; no history of nephritic syndrome, $\leq$ one positive urine dipstick reading, and proteinuria less than 150 mg/d; no severe uncontrolled hypertension; and no history of venous or arterial thromboembolic events. The trial was approved by the local ethics committee. Written informed consent was required.

Study Treatment

Patients received bevacizumab (Avastin; Roche, Basel, Switzerland) 10 mg/kg intravenously every 14 days in combination with cyclophosphamide (Endoxan; Baxter, Deerfield, IL) 50 mg one tablet daily at 9:00 AM, plus capecitabine (Xeloda; Roche) 500 mg one tablet thrice daily after meals. Cycles were repeated every 14 days. Bevacizumab was to be permanently discontinued in patients who developed GI perforation, wound dehiscence requiring medical intervention, serious bleeding, a severe arterial thromboembolic event, nephrotic syndrome, or hypertensive crisis. Temporary suspension of bevacizumab was recommended in patients with evidence of moderate to severe proteinuria pending further evaluation and in patients with severe hypertension that was not controlled with medical management. Cyclophosphamide and capecitabine were reduced in case of grade ≥ 2 hematologic toxicity, cystitis, GI toxicity, or hand-foot syndrome. To achieve a 50% dose reduction, cyclophosphamide was administered as one 50-mg tablet every other day, and capecitabine was administered as one 500-mg tablet once daily alternated with one 500-mg tablet twice daily.

Study Evaluations

The baseline evaluation included complete history and physical examination, assessment of performance status, CBC and differential, metabolic profile, coagulation studies, ECG, urine dipstick, and serum pregnancy test in women of childbearing age. Baseline staging was performed with computed tomography scans of the head, chest, and abdomen. Response to treatment was evaluated every four treatment cycles using Response Evaluation Criteria in Solid Tumors.[14] Toxicities were graded using the National Cancer Institute Common Terminology Criteria of Adverse Events (version 3; http://ctep.cancer.gov/forms/CTCAEv3.pdf).

Biologic Correlates

The number and viability of CECs and CEPs were measured on days 1, 14, and 56 by six-color flow cytometry, as previously described.[15] Transmission electron microscopy and polymerase chain reaction studies were performed in parallel in sorted CECs that were confirmed to be of endothelial nature by the observation of Weibel-Palade bodies and the finding of high levels of RNA expression of the endothelial-specific gene VE-cadherin (Mancuso et al, submitted for publication). CECs were enumerated as DNA-positive, $CD45^-$, $CD31^+/CD146^+$ cells. CEPs were enumerated as DNA-positive, $CD45^-$, $CD133^+/CD34^+$ cells. CEC subpopulations expressing VEGFR1, VEGFR2, or VEGFR3 were also enumerated.

Statistical Analysis

The primary aim of this study was to assess the activity of a metronomic regimen with oral cyclophosphamide and capecitabine plus bevacizumab in terms of overall CB, which was defined as the objective RR (complete response [CR] and partial response [PR]) plus the rate of stable disease (SD) at 24 weeks after treatment initiation.

To allow early termination of the trial in case of inactive treatment, a Simon two-stage optimal design was used, minimizing the expected sample size given a poor CB. If a proportion of patients achieving a CB of $\leq 40\%$ was observed for the proposed regimen, then the study would be closed. In contrast, a proportion of 60% or higher would suggest that the regimen is effective and worthy of additional study. At the first stage, 16 patients were accrued. If eight or more patients obtained a CB among this cohort, the trial would be extended, and 30 additional patients would be enrolled, to reach a total of 46 patients. At the second stage, if 24 or more patients obtaining a CB were observed among the total of 46 patients, the treatment would be considered worthy of additional study. This two-stage design yielded a power of 80% to detect a true RR of 60% or greater. The type I error was set at 5%. The variability of the CB rate and overall RR was assessed by calculating the exact 95% CI. Secondary end points included progression-free survival (PFS), the determination of toxicity of the regimen, and the prognostic value of circulating markers of angiogenic activity.

Time to disease progression (TTP) was calculated from the start of treatment and evaluated using the Kaplan-Meier estimates of the survival curves. The log-rank test was used for the comparisons of survival curves between subgroups of patients.

The Fisher's exact test and the Wilcoxon rank sum test were used to evaluate differences between responders and nonresponders in the distribution of categoric and continuous variables, respectively, and the log-rank test was used to evaluate the prognostic value of variables on PFS. Subgroups

analyses and/or analyses of secondary end points were exploratory in nature. All *P* values are two-sided.

RESULTS

A total of 47 patients were enrolled. Forty-six patients were assessable for response and toxicity (one patient did not receive study treatment as a result of worsening of clinical conditions), and 40 patients were assessable for CB; it was too early to assess CB in six patients. Patient characteristics at baseline are listed in Table 1.

Of 46 patients assessable for response, we observed one CR (2%), 21 PRs (46%), 19 patients (41%) with SD, and five patients (11%) with progression of disease during treatment, for an overall RR of 48% (95% CI, 33% to 63%). Of 40 patients in whom the period of observation and treatment was longer than 24 weeks, we observed eight additional long-term disease stabilizations (SD > 24 weeks), for an overall CB rate (one CR + 18 PRs + eight SDs ≥ 24 weeks) of 68% (95% CI, 51% to 81%). Median TTP was 42 weeks (95% CI, 26 to 72 weeks; Fig 1). Median number of cycles per patient was 13 (range, two to 34 cycles).

Treatment was well tolerated. Main adverse effects are listed in Table 2. Grade 3 or 4 adverse effects included hypertension (n = 8), leukopenia (n = 2), neutropenia (n = 2), transaminitis (n = 2, both with liver metastases), proteinuria (n = 1), nausea (n = 1), and vomiting (n = 1). Hypertension was manageable with adequate therapy. In 14 patients, monotherapy was prescribed to control blood pressure (grade 2), whereas in eight patients, more than one drug was required (grade 3); however, no hypertensive crises were observed in the course of the trial. Proteinuria (any grade) was observed in 15 patients. Of the 46 patients who were enrolled onto the study, 23 were withdrawn as a result of disease progression, two patients refused to continue treatment within the trial, and only one patient was withdrawn from the study as a result of adverse effects (nephrotic syndrome).

At univariate analysis, hormone receptor status was significantly related to CB. CB rate was 86% (12 of 14 patients) in patients with both estrogen receptor (ER)–positive and progesterone receptor–positive disease, 71% (12 of 17 patients) in patients with ER-positive and progesterone receptor–negative disease, and 33% (three of nine patients) in patients with endocrine nonresponsive disease ($P = .04$). Median TTP was 49 weeks in the ER-positive cohort and 19 weeks in the ER-negative cohort ($P = .002$; Fig 2).

Biologic Results

Baseline (before treatment) CEC count was significantly increased in patients who subsequently had a clinical response ($P = .02$; Fig 3A) and in patients who subsequently had a CB ($P = .01$; Fig 3B) compared with patients who did not. Similarly, viable CECs at baseline were significantly increased in patients who subsequently had a clinical response ($P = .02$, data not shown) as well as in patients who achieved a CB ($P = .03$, data not shown). Flow cytometry viability studies indicated that viable CEC count was correlated with the total CEC count (correlation index = 0.97, data not shown); a similar correlation was found between apoptotic CEC count and total CEC count (data not shown). Patients who had ≥ 0.27 apoptotic CECs/μL (ie, the 25th percentile distribution value) before the beginning of therapy had a significantly better PFS ($P = .04$; Fig 4). Patients with a

Table 1. Patient Characteristics at Baseline

Characteristic	No. of Patients	%
No. enrolled	47	
No. assessable	46	
Age, years		
Median	57.5	
Range	35-75	
Body weight, kg		
Median	65	
Range	45-99	
Menopausal status		
Premenopausal	15	33
Postmenopausal	31	67
Metastatic sites*		
Bone	16	35
Lung	14	30
Liver	21	46
Lymph nodes	19	41
Pleura	7	15
Other	6	13
No. of metastatic sites		
1	20	43
2	19	41
≥3	7	15
Tumor hormone receptor status†		
ER positive/PgR positive	15	33
ER positive/PgR negative	20	43
ER negative/PgR negative	11	24
HER-2/*neu* status		
Absent	17	37
1+	22	48
2+	6	13
3+	1	2
Prior neoadjuvant therapy		
None	35	76
CT	6	13
CT/HT	5	11
Prior adjuvant therapy		
None	11	24
CT	6	13
HT	10	22
CT/HT	19	41
Prior therapy for metastatic disease		
None	11	24
CT	3	7
HT	16	35
CT/HT	14	30
CT/HT/trastuzumab	2	4
No. of prior metastatic CT regimens		
0	27	59
1	11	24
≥ 2	8	17
Prior anthracycline	29	63
Prior taxane	11	24

Abbreviations: ER, estrogen receptor; PgR, progesterone receptor; CT, chemotherapy; HT, hormone therapy.
*Multiple sites possible.
†Positive: ≥ 10%.

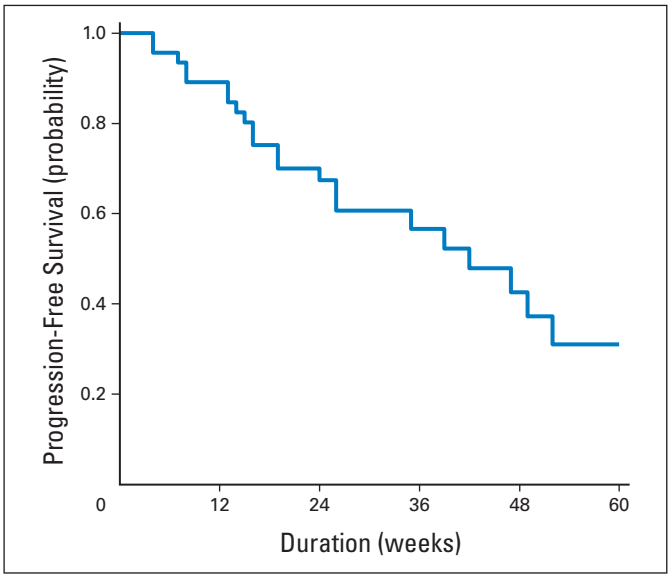

Fig 1. Progression-free survival (Kaplan-Meier method).

CB and a clinical response also showed a trend towards a reduction in CEC count during therapy (Fig 3). The number and kinetics of CEP and of CEC subpopulations expressing VEGFR1, VEGFR2, or VEGFR3 did not significantly correlate with the clinical outcome.

DISCUSSION

MBC is a chronic disease requiring specific strategies to control the disease progression and related symptoms.[16] The treatment choice is often based on evidence obtained from trials designed to investigate therapy-related issues such as whether one treatment yields better responses or longer TTP. However, few trials test treatment strategies based on the minimal burden of adverse effects necessary for reasonable control of disease.

The vascular-targeted therapy approach is designed to interfere with new vessel formation, thereby slowing or preventing tumor growth. Targeted antiangiogenic drugs induce long-term changes in the tumor vasculature and are designed for continuous treatment. Similarly, chronic administration of lower doses using more frequent administration schedules of cytotoxics, without protracted breaks (metronomic delivery), has been tested to optimize the antiangiogenic effects and minimize toxicity.[17,18] Preventing repair of affected tumor vessels, by such mechanisms as mobilization and incorporation of CEPs, is part of the rationale for the more frequent schedule of drug administration.[19] Metronomic low-dose chemotherapy is increasingly recognized as a useful tool for the treatment of several types of cancer, such as hormone-refractory prostate cancer,[20] heavily pretreated sarcoma,[21] melanoma,[22] ovarian cancer,[9] and breast cancer.[23] The clinical outcomes of antiangiogenic treatments are likely to be quite distinct from those seen with conventional cytotoxic therapies because inhibition of tumor progression with long-term stabilization of cancer, rather than rapid destruction of existing disease, may be anticipated. However, one of the most desirable end points of treatment for the individual patient with MBC is represented by the achievement of prolonged disease control (eg, CB), and strategies that can induce a CB represent an appropriate therapeutic choice.

Table 2. Main Adverse Effects

Adverse Effect	No. of Patients	
	All Grades	≥ Grade 3
Mucositis	36	—
Leukopenia	25	2
Asthenia	25	—
Nausea	24	1
Hand-foot syndrome	24	—
Hypertension	22	8
Neurology (paresthesias)	20	—
Neutropenia	15	2
Constipation	17	—
Transaminitis	17	2
Headache	16	—
Diarrhea	16	—
Proteinuria	15	1
Gastritis	11	—
Bleeding	10	—
Anemia	8	—
Vomiting	8	1
Cystitis	7	—
Skin	7	—
Thrombocytopenia	7	—
Arthralgia	6	—
Hot flashes	4	—
Nail changes	4	—
Sweating	3	—
Alopecia	2	—
Fever	2	—
Loss of appetite	2	—
Abdominal pain	2	—
Myalgia	1	—
Hemorrhoids	1	—
Conjunctivitis	1	—

This is the first report focusing on the combination of metronomic capecitabine and cyclophosphamide combined with bevacizumab in patients with MBC. The results indicate that a clinically relevant fraction of MBC patients (68%) achieved a control of the disease for at least 6 months with this treatment strategy. Our results are in line with those achieved with other schedules of chemotherapy administered either alone or in combination with bevacizumab in patients receiving first-line treatment for MBC. In a recently published phase III trial,[24] patients were randomly assigned to either paclitaxel plus bevacizumab or paclitaxel alone. The objective RR was 36.9% *v* 21.2% for paclitaxel plus bevacizumab versus paclitaxel alone, respectively ($P < .001$). These results also compare favorably with two previously reported experiences with metronomic chemotherapy alone, where CB rates of 31.7%[3] and 41.5%[4] were achieved. In the present study, a new schedule was designed to combine capecitabine and cyclophosphamide. This schedule has shown some confirmation of experimental efficacy. In animal models, combination therapy of capecitabine and cyclophosphamide showed synergistic antitumor activity without significant toxicity.[25] Both drugs demonstrated enhanced clinical activity if combined with bevacizumab.[8,12] Moreover, another fluorouracil prodrug (uracil-ftorafur), when administered in

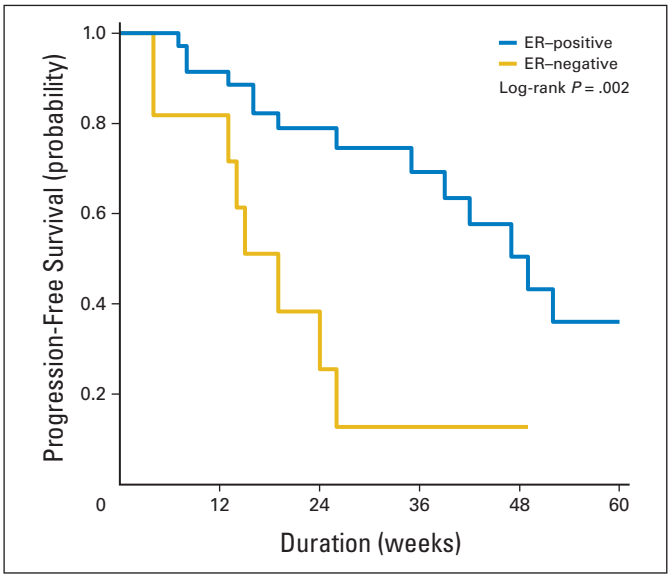

Fig 2. Progression-free survival according to estrogen receptor (ER) status.

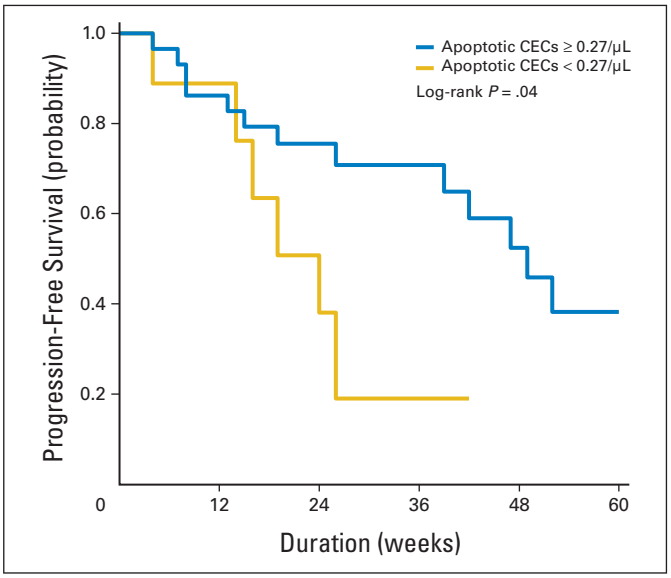

Fig 4. Progression-free survival according to apoptotic circulating endothelial cells (CECs) at baseline.

a daily low-dose manner with daily low-dose cyclophosphamide, produced significant antitumor effects in a new model of advanced MBC.[13]

In this study, hormone receptor status was significantly related to CB. These results are in line with previously reported data in MBC, in which endocrine-responsiveness was related to better PFS and survival.[26] We previously showed that the probability of prolonged CB with metronomic therapy was higher in endocrine-responsive MBC.[23] These results might be related to the biology of endocrine-responsive disease, which is characterized by indolent, low-proliferating tumors and, therefore, more likely to have prolonged stabilization.[27] Moreover, there is a biologic rationale for improved activity of antiangiogenic treatment in endocrine-responsive tumors.[28] Several growth factors influence proliferation and survival of ER-positive hormone-resistant disease.[29] In particular, VEGF is elevated in patients with endocrine-responsive disease who do not respond to hormone therapy, therefore contributing to disease progression and resistance to endocrine therapies.[30-32]

It is noteworthy that the CB was achieved without significant acute or delayed toxicity. In the present study, there was limited evidence of adverse effects related to classic direct cytotoxic effects (eg, significant myelotoxicity or alopecia). In fact, only 4% of the patients had grade greater than 2 leukopenia or neutropenia, and only 4% of the patients had some hair loss (Table 2). The adverse effects related to bevacizumab were, as previously reported, manageable and reversible when the treatment was stopped.

The identification of patients who might benefit from targeted antiangiogenic therapies is crucial for the optimization of the treatment strategy. In the present study, we found that the baseline CEC count is a predictive biomarker of outcome that might be useful for the selection of advanced breast cancer patients who would be candidates for metronomic chemotherapy and bevacizumab. Flow cytometry

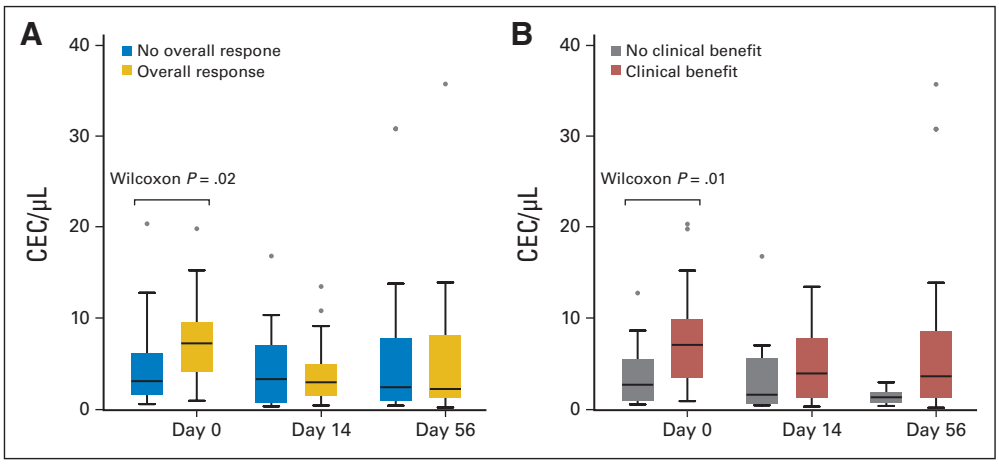

Fig 3. Circulating endothelial cells (CECs) at baseline, day 14, and day 56, according to (A) overall response and (B) clinical benefit. Whiskers (standard span) were extended to 1.5× the interquartile range outside of the first and third quartile. Outliers beyond the standard span are indicated by gray circles.

studies have indicated that CECs are significantly increased in untreated cancer patients compared with healthy controls. We previously showed that, in patients with advanced breast cancer receiving metronomic chemotherapy, an increased CEC count after 2 months of therapy was a good predictor of disease-free and overall survival.[33] An increased CEC count has already been reported in cancer-bearing animals treated with some antiangiogenic drugs.[34,35] Previous clinical studies measured CECs during or after therapy and not at baseline as in the present study. We showed that baseline and viable CECs were significantly increased in patients who had a clinical response and in patients who achieved a CB. Moreover, patients who had a baseline increased apoptotic CEC count had a significantly better PFS. Flow cytometry viability studies indicated that apoptotic CEC count was related to total CEC count (data not shown). Therefore, the baseline total, viable, and apoptotic CEC count might represent an indirect measure of the angiogenic turnover and an indicator of better response to antiangiogenic therapy, supporting the use of these treatments in patients expressing high levels of baseline CECs. Further prospective trials are required to confirm the value of these data in patients who are candidates for antiangiogenic agents. If confirmed, future selection of antivascular agents should also be based on the CEC count before treatment.

In conclusion, the results of this study indicate that metronomic capecitabine and cyclophosphamide combined with bevacizumab provide long-term disease control in a high proportion of patients, without significant toxicity despite prolonged use. The low burden in terms of personal costs to the patient and the possibility of continuing the treatment for up to several months in responders, as is often required in advanced breast cancer patients, support this regimen as an additional therapeutic tool in MBC patients.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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