# Prolonged clinical benefit with metronomic chemotherapy in patients with metastatic breast cancer

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**The clinical efficacy and antiangiogenic effect of low-dose, metronomic administration of cyclophosphamide (CTX) and methotrexate (MTX) (CM) have been demonstrated. The authors report results and long-term follow-up for patients with metastatic breast carcinoma who obtained prolonged clinical benefit with CM. Prospectively collected data from two successive clinical trials were evaluated. From July 1997 to October 2003, patients with metastatic breast carcinoma were treated with low-dose oral chemotherapy (MTX 2.5 mg, twice daily on day 1 and day 2 or 4, and CTX 50 mg daily). Patients who achieved prolonged clinical benefit for a duration of 12 months or more (complete remission, partial remission or stabilization of disease) were considered for the analysis. Median follow-up was 23 months. A total of 153 patients were enrolled and are evaluable: Eastern Cooperative Oncology Group performance status 0–1 in 90 patients, two or more sites of metastatic disease in 97 patients, zero regimen for metastatic breast carcinoma in 48 patients. Among 153 patients, five demonstrated complete remission and 25 partial remission. The proportion of patients who achieved prolonged clinical benefit was 15.7% (95% confidence interval 9.9–21.4%). Median time to progression for patients with prolonged clinical benefit was 21 months (range 12–37+ months). One patient maintained complete remission 42 months after therapy discontinuation. At the multivariate analysis endocrine responsiveness and the achievement of an objective response significantly correlated with the achievement of prolonged clinical benefit. Metronomic chemotherapy can induce prolonged clinical benefit in metastatic breast cancer, supporting its role as an additional therapeutic tool in the treatment of patients with metastatic breast carcinoma. *Anti-Cancer Drugs* 17:961–967 © 2006 Lippincott Williams & Wilkins.**

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## Introduction

Metastatic breast cancer is a chronic disease requiring specific strategies to control disease progression and related symptoms. Treatment can assure a significant prolongation of survival, symptomatic control and maintenance of quality of life [1,2]. 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 time to progression. Very few trials test treatment strategies, causing minimal burden of side-effects necessary for reasonable control of disease.

A number of recent preclinical and clinical studies have exploited conventional chemotherapeutic drugs as angiogenesis inhibitors [3]. Chronically, administration of lower doses and more frequent schedules of cytotoxics (metronomic delivery) have been tested, in order to optimize the antiangiogenic effects, to minimize toxicity and overcome the repair of endothelial cells [4,5]. In fact, Browder *et al.* [6] have shown that chemotherapeutic drugs given at the maximum tolerated dose (MTD) can cause apoptosis of tumor-associated vessels in ectopically growing mouse tumors, but this damage can be repaired rapidly during the prolonged recovery periods necessary for myeloid recovery following MTD chemotherapy. Hence, by giving chemotherapy more frequently, daily, weekly or twice weekly, the endothelial cells' repair process can be compromised and the potential effects of chemotherapy enhanced [6].

The availability of low-toxic and easily delivered regimens that may be administered for a longer duration than common cytotoxic therapies, without significant toxicity, could provide the individual patient choices for effective therapy, while allowing her to conduct her life as close to her expectations as possible. In two previous trials, we observed a response rate of 19 and 20.9% with low-dose

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cyclophosphamide (CTX, 50 mg/daily) plus methotrexate (MTX, 5 mg/daily twice a week) [7,8].

In order to define a possible role of metronomic chemotherapy in inducing prolonged disease control and in order to identify factors predictive of clinical benefit, we analyzed our prospectively collected data on patients with metastatic breast carcinoma treated at the European Institute of Oncology between July 1997 and January 2003 who participated in two trials of metronomic chemotherapy. We report the complete response (CR), partial response (PR) and stabilization of disease (SD), maintained for at least 12 months for the entire patient population and subsets according to prognostic factors.

## Patients and methods

From July 1997 to January 2003, the Division of Medical Oncology at the European Institute of Oncology carried out two successive protocols that incorporated metronomic chemotherapy to treat patients with histologically confirmed metastatic breast cancer. In the first study, 63 patients were treated with MTX orally at a dose of 2.5 mg twice a day on days 1 and 2 every week (10:00, 17:00 h) and CTX orally at a dose of 50 mg a day (09:00 h). In the second study, 90 patients received MTX orally at a dose of 2.5 mg twice a day on days 1 and 4 every week (10:00, 17:00 h) and CTX orally at a dose of 50 mg a day (09:00 h). The two studies were approved by the Ethical Committee. Each patient included in these studies gave a written informed consent.

Patients included in the studies were required to have histologically confirmed metastatic breast carcinoma either pretreated or not pretreated. Other inclusion criteria were measurable or evaluable disease, age $\leq 80$ years, Eastern Cooperative Oncology Group performance status $< 3$, adequate bone marrow reserve defined as white blood cells $> 4000\,mm^3$ and platelets $> 100\,000\,mm^3$, adequate renal function (serum creatinine $< 120\,\mu mol/l$) and hepatic function [serum bilirubin $< 20\,\mu mol/l$, AST (SGOT) $< 60\,IU/l$], and signed informed consent. Baseline evaluation included clinical examination, chest X-ray, liver ultrasound or computed tomography (CT) scan, bone nuclear scan, electrocardiogram, complete biochemical and hematological tests. Complete blood count was then repeated every 14 days and biochemical tests were repeated every 28 days. Toxicity was evaluated according to the National Cancer Institute Common Toxicity Criteria (version 3.0) by clinical and laboratory investigation.

Assessment of response was performed according to the World Health Organization criteria after every 2 months of therapy. CR was defined as the disappearance of all known lesions on two separate measurements at least 4 weeks apart. PR was defined as a reduction of each lesion by at least 50%. SD was defined as a decrease of less than 50% or an increase of less than 25% with no new lesions, and progressive disease (PD) as an increase of more than 25% or appearance of new lesions. In case of CR or SD, therapy continued until progression or unacceptable toxicity. Prolonged clinical benefit (PCB) was defined as the proportion of patients who achieved no disease progression (CRs, PRs or SDs) for at least 12 months. The duration of response was measured from the date of achievement of response to the date of disease progression. Time to progression was defined as the length of time from the start of treatment to the date of disease progression. Overall survival was defined as the time from the start of treatment to the date of death (from any cause) or the date of last follow-up. The following clinical and biological parameters related to the long responder patients were analyzed: age, performance status, hormone receptors status (performed with immunohistochemistry at the European Institute of Oncology for all the patients), presence of progressive disease at study entry, pre-treatment for metastatic disease, sites of metastasis, number of sites, baseline vascular endothelial growth factor (VEGF) and its modifications. Serum VEGF was determined at baseline and every month thereafter as previous described [7].

### Statistical analysis

The main aims of this study were to estimate the percentage of PCB, defined as a time to progression of at least 12 months, obtained with metronomic chemotherapy, and to identify the clinical and pathological features that predicted a prolonged benefit. Associations between categorical variables were assessed by Fisher's exact test and by the $\chi^2$-test for trend in proportions in case of ordered qualitative categories. Differences in quantitative variables between groups were evaluated by the Wilcoxon rank-sum test. In order to assess which factors independently predict a PCB, multiple logistic regression was conducted. The variables included in the multiple regression are age, number of previous lines of chemotherapy for advanced disease (a categorical variable with three levels: zero, one and more than one previous line), achievement of an objective response, previous progressive disease at study entry, number of sites of disease, site of disease (a categorical variable with three levels: soft tissue only, bone with or without soft tissues, visceral involvement with or without other sites), expression of hormone receptors (estrogen and progestin receptors negative, defined as lower than 1% of tumor cells positive at immunohistochemical assessment vs. estrogen and/or progestin receptors positive in 1 or more of tumor cells). Models explored included those with all baseline factors, with and without the two-way interactions, as well as all models including two baseline factors and the two-way interactions among them. The likelihood ratio test was used to assess the contribution of individual variables or groups of variables. A backward

selection was performed, with 0.05 significance level for acceptance of terms. Odds ratios with 95% confidence intervals (CIs) and *P* values are reported. Time-to-event endpoints were estimated using the product-limit method of Kaplan and Meier. Disease-free survival and overall survival were calculated from the beginning of treatment to the date of disease progression and the date of death for any cause, respectively. The duration of response was measured from the date of documentation of an objective remission to the date of disease progression. Statistical analysis was performed with *R* [9].

## Results

On 153 evaluable patients (Table 1), oral CTX plus MTX produced five CRs and 25 PRs, providing an objective response rate of 19.6% (95% CI, 13.3–25.9%). Twenty-six patients had disease stabilization for 24 weeks or longer, for an overall clinical benefit of 36.6% (95% CI, 29.0–44.2%). The median follow-up was 23 months (range, 1.5–44.6 months).

Twenty-four patients (15.7%; 95% CI, 9.9–21.4%) achieved a PCB [no disease progression (CRs, PRs or SDs) for at least 12 months] and were the subject of the present analysis. A CR was achieved in five patients, PR in 11 patients and SD in eight patients. Characteristics of the patients are listed in Table 1.

In patients with PCB, median time to response was 3 months (range, 1–10 months), median duration of response 18 months (range, 8–32 + months) and median time to progression 21 months (range, 12–37 + months). Median survival has not yet been reached. Median duration of treatment was 20.4 months (range, 12–34 months). One patient with histologically confirmed lung metastasis achieved CR of disease after 10 months of treatment and remained in CR after 63 months from the diagnosis of metastasis. In this patient, therapy was stopped after 1 year and 7 months due to possible long-term toxicity. Figure 1 shows the baseline chest CT scan, the CT scan performed after 3 months (first response evaluation) and the CT scan after 4.5 years from treatment start. Moreover, in five patients we registered a control of disease for 24 months or longer, with two patients who had progression of disease after 26 and 36.8 months, and three patients who are progression-free after 24.7, 34.6 and 30.1 months, respectively, from the start of therapy. Among these patients, one stopped the therapy without evidence of progression of disease after 34 months because of potential long-term toxicity.

Higher percentages of tumors were classified estrogen and progesterone receptor-positive (75 vs. 48.8%; $P < 0.03$) in the group of patients who achieved PCB than in the other patients. Moreover, the achievement of objective response (CR and PR) is correlated with the likelihood to obtain long-term benefit (66.6 vs. 10.9%, $P < 0.001$). The proportion of PCB was higher in patients receiving metronomic therapy as first-line chemotherapy for advanced disease (25%) than in those pretreated with one (13.6%) or more lines (8.7%) of chemotherapy ($P = 0.02$). Finally, there was a trend towards a higher chance of PCB in tumors with low (below 20%) Ki-67 labeling index (31.6%) compared with those with high (20% or above) Ki-67 (10.9%), which did not reach statistical significance ($P = 0.07$) (Ki-67 assessable in 74 patients). HER2/*neu* status did not predict PCB ($P = 1$).

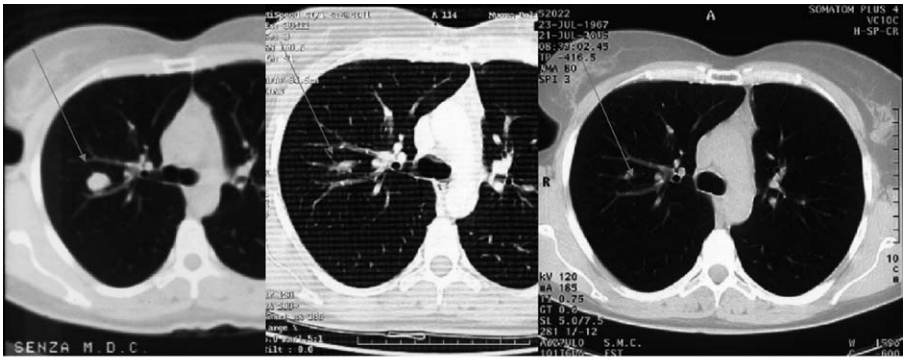
We reviewed the body surface area of each patient with PCB in order to evaluate whether the metronomic treatment based on fixed doses of CTX and MTX could induce differences in the cumulative dose according to this parameter, thus influencing the response. We found a wide range in body surface area values (1.4–1.97) with a median value of 1.62 in PCB patients.

**Table 1 Features of PCB and non-PCB patients**

| Patients | PCB patients (*n*/%) | Others (*n*/%) | *P* value |
|---|---|---|---|
| Entered | 24 | 129 | |
| Median age (years) (range) | 54.8 (34–76) | 54.5 (34–81) | 0.63[a] |
| Baseline ECOG performance status | | | |
| 0 | 14/58.3 | 64/49.6 | 0.68[b] |
| 1 | 1/4.1 | 11/8.5 | |
| Unknown | 9/37.5 | 54/41.9 | |
| PD at study entry | | | |
| No | 5/20.8 | 36/27.9 | 0.62[b] |
| Yes | 19/79.2 | 93/72 | |
| Chemotherapy for advanced disease | | | |
| None | 12/50 | 36/27.9 | 0.02[c] |
| 1 line | 8/33.3 | 51/39.5 | |
| 2 or more lines | 4/16.7 | 42/32.5 | |
| Number of sites | | | |
| 1 | 8/33.3 | 48/37.2 | 0.41[c] |
| 2 | 13/54.2 | 41/31.8 | |
| 3 + | 3/12.5 | 40/31 | |
| Tumor main site | | | |
| Soft tissues | 2/8.3 | 26/20.1 | 0.41[c] |
| Bone | 6/25 | 20/15.5 | |
| Viscera | 16/66.7 | 83/64.3 | |
| ER and PgR-negative | 4/16.7 | 51/39.5 | 0.03[b] |
| ER and/or PgR-positive | 18/75 | 63/48.8 | |
| Unknown | 2/8.3 | 15/11.6 | |
| Ki-67 | | | |
| < 20% | 6/25 | 13/10 | 0.07[b] |
| ≥ 20% | 6/25 | 49/38 | |
| Unknown | 12/50 | 67/51.9 | |
| Her2/*neu* | | | |
| 0/ + / + + | 10/41.7 | 35/27.1 | 1[b] |
| + + + | 2/8.3 | 9/7 | |
| Unknown | 12/50 | 85/65.9 | |
| Response | | | |
| CR | 5/20.8 | 0/0 | < 0.001[c] |
| PR | 11/45.8 | 14/10.9 | |
| SD | 8/33.3 | 48/37.2 | |
| PD | – | 67/52 | |

PCB, prolonged clinical benefit; ECOG, Eastern Cooperative Oncology Group; CR, complete remission; PR, partial remission; SD, stabilization of disease; PD, progressive disease; ER, estrogen receptor; PgR, progesterone receptor.
[a]Wilcoxon rank sum test.
[b]Fisher exact test.
[c]Test for trend.

**Fig. 1**




Lung metastasis at baseline (a), after 3 months of treatment (b) and after 4.5 years from the start of treatment.

**Table 2 Serum VEGF concentrations (pg/ml); mean ± SD at baseline and 2 months after treatment according to clinical benefit**

| | PCB ($n=20$) | No PCB ($n=98$) |
|---|---|---|
| Baseline | 364.5 ± 241.5 | 426.0 ± 357.5 |
| 2 months | 266.6 ± 183.4 | 340.2 ± 269.5 |
| Difference | − 97.9 ± 178.2 (26.9% reduction) | − 85.8 ± 236.2 (20.1% reduction) |
| Signed-rank | $P=0.007$ | $P<0.0001$ |

VEGF, vascular endothelial growth factor; PCB, prolonged clinical benefit.

The mean VEGF level decreased with treatment from 364.5 (± 241.5) at baseline to 266.6 (± 183.4) at 2 months, with a mean reduction of 97.9 (± 178.2) in the PCB group ($P = 0.007$) and from 426.0 (± 357.5) at baseline to 340.2 (± 269.5) at 2 months, with a mean reduction of 85.8 (± 236.2) in the other group ($P < 0.0001$) (Table 2). No statistically significant difference in reduction of VEGF levels between the two groups was registered. A trend to a higher VEGF reduction, however, was observed in the group of patients who achieved PCB (26.9% reduction) when compared with patients with no PCB (20.1% reduction). We have not found differences in the proportion of PCB patients between the two schedules of MTX administration (data not shown).

In the multivariate logistic regression, the achievement of an objective response and the presence of hormone receptors were the only two factors that remained statistically significant in the model to predict PCB. Patients obtaining an objective response (CR or PR) were 14.98 times (95% CI, 4.97–45.14, $P < 0.001$) more likely to achieve PCB than patients without objective response. After exclusion of 67 patients showing PD at first evaluation of response (after 2 months of therapy), patients who obtained an objective response (CR or PR) at any time were 6.13 times (95% CI, 1.96–19.17, $P = 0.002$) more likely to achieve a PCB than patients with stable disease. Patients with estrogen and/or progesterone-positive tumors were 3.69 times (95% CI, 1.02–13.40, $P = 0.045$) more likely to achieve a PCB than patients with estrogen and progesterone-negative tumors.

Prolonged treatment was well tolerated and side-effects were mild, as previously reported [6,7]. The most frequently encountered toxicity was grade I–II leukopenia, which was observed in 54% of the cases. Increases in transaminase values were registered in 12 cases, one patient with grade 3 toxicity (Table 3). A complete recovery of the functions was achieved with either reduction or transient interruption of MTX. No side-effects related to prolonged administration of therapy (e.g. cystitis) were observed.

## Discussion

A dose-related cytotoxic effect on tumor cells has been postulated in the past as the main cause of anti-neoplastic efficacy using chemotherapy for the treatment of breast cancer. The principle of dose intensity is supported by experimental model systems in which a slight increase in drug dose may result in a large addition in tumor cell kill [10–13]. The issue of dose intensity and efficient tumor kill in advanced breast cancer is, however, controversial [14–16] and still a question for further studies. Interactions between drugs, tumor cells, hormones (and endocrine organs) and stroma are, however, very complex [17], and the action of low doses of common chemotherapeutic agents on other compartments, mainly the vasculature, was recently demonstrated [18]. Recent publications on in-vitro activity of taxanes and vinca alkaloids at chronic, low-dose exposure that resulted in inhibiting vessel formation and growth [19,20] support the concept that the more frequent pace of administration might be important in conferring efficacy to this schedule of chemotherapy.

**Table 3 Side-effects on 24 prolonged clinical benefit patients**

| Side-effect | 1 | | 2 | | 3 | |
|---|---|---|---|---|---|---|
| | Number | Percentage | Number | Percentage | Number | Percentage |
| Leukopenia | 7 | 29 | 6 | 25 | 2 | 8 |
| Neutropenia | 7 | 29 | 1 | 4 | 2 | 8 |
| Thrombocytopenia | 0 | – | 0 | – | 1 | 4 |
| Anemia | 3 | 13 | 2 | 8 | 0 | – |
| Alopecia | 1 | 4 | 0 | – | 0 | – |
| Nausea/vomiting | 10 | 41 | 1 | 4 | 0 | – |
| Gastric pain | 1 | 4 | 1 | 4 | 0 | – |
| Mucositis | 4 | 17 | 1 | 4 | 0 | – |
| Transaminases | 4 | 17 | 7 | 29 | 1 | 4 |
| Diarrhea | 1 | 4 | 0 | – | 0 | – |
| Stypsis | 1 | 4 | 0 | – | 0 | – |
| Fever | 1 | 4 | 2 | 8 | 0 | – |
| Skin | 1 | 4 | 0 | – | 0 | – |
| Infection | 0 | – | 1 | 4 | 0 | – |
| Astenia | 4 | 17 | 1 | 4 | 0 | – |

The objective of antiangiogenic therapies, or better of therapies aimed at impeding the vascular supply of tumors, 'vascular-targeted therapy', is to interfere with new vessel formation, thereby preventing tumor growth and limiting metastatic potential. Antiangiogenic drugs induce long-term changes in the tumor vasculature (mostly maturation) and are designed for continuous treatment. The clinical outcomes are therefore likely to be quite distinct from those seen with conventional cytotoxic therapies, as inhibition of tumor progression with long-term stabilization of cancer, rather than destruction of existing disease, may be anticipated [21]. In view of the palliative goal of treatment in metastatic breast cancer, symptomatic control with maintenance of quality of life represents a relevant clinical outcome and one of the most desirable endpoints of treatment for the individual patient, and strategies that can induce a prolonged disease control (e.g. PCB) represent an appropriate therapeutic choice.

This is the first report focusing on outcome after long-term follow-up of patients with metastatic breast carcinoma treated with metronomic chemotherapy in a single institution. The presented results indicate that a clinically relevant fraction of metastatic breast carcinoma patients achieve long-term clinical benefit and survival with this treatment option.

On 153 evaluable patients, a disease control lasting more than 12 months (PCB) was registered in 24 patients (15.7%) with six patients who presented a control of disease for 24 months or longer. As previously shown, one patient is alive with CR of disease after 63 months from diagnosis of lung recurrence.

It is noteworthy that the PCB was achieved without significant delayed toxicity [7,8]. In fact, some important aspects about the chronic use of CTX (and MTX) in patients treated for several months should be raised. CTX given together with MTX and fluorouracil has been reported to slightly increase the incidence of leukemia [22]. Dealing with leukemic risk while discussing treatment for advanced breast cancer might be futile; however, no evidence of bone marrow disorders (such as abnormal white blood cells count, anemia or thrombocytopenia) was seen. Changes of transitional epithelium [23] and immunosuppression [24,25] are also associated with prolonged use of CTX. While instructions for an abundant fluid intake evade the former, a pause in the administration of the drugs could have been hypothesized. With continuous exposure to the therapy for a median of 20.4 months, however, we encountered neither of these, indicating for the first time that metronomic chemotherapy administered for a prolonged time is feasible with occurrence of these expected side-effects.

The identification of patients who might benefit from metronomic chemotherapy is crucial for the optimization of the treatment strategy. At this stage, the disease is heterogeneous with respect to patient characteristics such as age, performance status, co-morbidities, prior adjuvant therapies and tumor features such as disease-free interval, site and extension of metastases, hormonal sensitivity, and HER2 status. In this study, the expression of steroid hormone receptors was observed in a larger percentage of patients who achieved PCB than in the other cohort of patients. These results are in line with previously reported data. A recently published analysis on 1581 treated with anthracycline-containing regimen has reported the correlation between estrogen receptor-positive breast carcinoma and better progression-free survival and overall survival when compared with estrogen receptor-negative tumors or unknown tumors [26]. The probability of PCB might be related to a peculiar biology of endocrine-responsive disease, with indolent tumors more likely to have prolonged stabilization. It has been shown that tumors that do not contain estrogen receptors are characterized by tumor cells that are rapidly

proliferating and significantly correlated with high replicative indexes and high tumor grade [27].

In our series, a significant correlation between the achievement of objectives response and subsequent PCB has been found, as previously showed in other retrospective analyses mainly conducted in metastatic breast cancer patients treated with high-dose chemotherapy and autologous hematopoietic stem cell support [28,29]. We also found a correlation between the number of previous treatments for advanced disease and probability of PCB. In fact, in our series, the proportion of PCB was particularly high in patients untreated with chemotherapy for advanced disease, although 50% of patients who achieved PCB were pretreated with chemotherapy for metastatic disease. The lower efficacy of metronomic chemotherapy in pretreated patients might be related to acquired resistance. The redundancy of angiogenic factors, presence of compensatory responses and vascular remodeling related to previous exposure to chemotherapy, as well as angiogenesis-independent tumor growth and increased hypoxia tolerance, might be hypothesized to explain a possible resistance to the metronomic approach [30,31].

VEGF is the ligand for the VEGF receptor 2 and has been recognized as a key potential target for the pharmacological inhibition of tumor angiogenesis. Several in-vitro and in-vivo studies indicated that values of VEGF can be reduced after treatment with agents inducing an antiangiogenic activity and that VEGF can be considered as a marker of the regulation of angiogenic factors. In a phase II study on refractory adult acute myelogenous leukemia, the administration of bevacizumab, an anti-VEGF monoclonal antibody, resulted in decreased VEGF values in 93% of the patients (67% undetectable) and marked decrease in microvessel density in marrow blasts [32,33]. We previously demonstrated that the magnitude of the VEGF reduction was related with the degree of response achieved [7,8]. Recently published results indicate that VEGF in the bloodstream is transported by blood cells, including leukocytes and platelets, and a correlation between VEGF value and platelet number has been observed [34]. In a subgroup of patients enrolled in our first trial [8] in which we tested both serum and plasma concentrations of VEGF, however, we showed a good correlation between serum and plasmatic values.

As showed in Table 2, two months after treatment, a decrease of VEGF level was observed both in patients who achieved PCB and in patients with no PCB, with a trend to a larger reduction for the PCB group although not statistically significant. The absence of a statistically significant difference in the present study in the VEGF drop between patients with PCB and those without PCB at 2 months might be related to the high percentage of patients (about 50%) without evidence of PD at 2 months in the latter group.

In conclusion, the results of this study indicate that low-dose, oral CTX and MTX provides long-term clinical benefit in a clinically significant proportion of patients, without significant toxicity, despite prolonged use.

The low burden in terms of personal costs to the patient and possibility to continue the treatment up to several years in responders, as often required in advanced breast cancer patients, support metronomic CTX plus MTX as an additional therapeutic tool in the population of metastatic breast cancer patients.

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