Cairo University

Journal of the Egyptian National Cancer Institute

www.nci.cu.adu.eg
www.sciencedirect.com

ORIGINAL ARTICLE

Metronomic chemotherapy in metastatic breast cancer: Impact on VEGF

Lobna R. Ezz El-Arab [a,*]**, Menha Swellam** [b]**, Manal M. El Mahdy** [c]

[a] *Radiation Oncology and Nuclear Medicine, Faculty of Medicine, Ain Shams University, Cairo, Egypt*
[b] *Department of Biochemistry, Genetic Engineering and Biotechnology Research Division, National Research Center, Dokki, Giza, Egypt*
[c] *Department of Pathology, Ain Shams University, Cairo, Egypt*

Abstract *Background:* Anticancer chemotherapy is thought to be effective by means of direct cytotoxicity on tumor cells. Alternative mechanisms of efficacy have been ascribed to several common anticancer agents; including cyclophosphamide (CTX) and capecitabine (Cap) when given at lower doses for prolonged period (metronomic chemotherapy) postulating an antiangiogenic activity as well.
Aim of work: To evaluate the action and tolerability of metronomic chemotherapy (MC) and its impact on serum vascular endothelial growth factor (VEGF) levels in metastatic breast cancer (MBC) patients.
Patients and methods: In this study we evaluated the clinical efficacy and tolerability of low dose, capecitabine (500 mg twice daily) together with oral cyclophosphamide (CTX) (a dose of 50 mg once daily) in patients with metastatic breast cancer. Vascular endothelial growth factor (VEGF), an angiogenic marker, was measured in the serum samples; at base line, and after 2 and 6 months of therapy.
Results: Sixty patients were evaluable. One achieved complete response (CR), 12 partial responses (PR), and 21 stable diseases (SD), while 26 were with progressive disease (PD). The overall response rate was 21.7% with overall disease control (CR, PR, and SD) 56.7%. The median time to progression

* Corresponding author. Address: Radiation Oncology and Nuclear Medicine, Faculty of Medicine, Ain Shams University, Lotfy El-Sayed St., Abbasia, Cairo, Egypt. Tel./fax: +20 226822670; mobile: +20 012 270 2185.
E-mail address: ezzlobna@yahoo.com (L.R. Ezz El-Arab).

Production and hosting by Elsevier

ELSEVIER

was 7 ± 2.59 months and overall survival 16 ± 8.02 months. Toxicity was mild, Palmar–plantar erythrodythesia was the most common side effect and was observed in 22 patients (37%), leucopenia (G1 + 2) was the most common hematological toxicity, and it was reported in 27% of the cases. The median VEGF level was significantly declined after 2 and 6 months of therapy compared to the base line among the patients with disease control (CR, PR, and SD). In multivariate logistic regression analysis, patients with post-menopausal, positive hormonal receptors, negative HER-2/Neu, and one metastatic site, were statistically significant and have a better disease control rate.

Conclusions: MC induced drop in VEGF, and was effective, minimally toxic regimen for the treatment of metastatic breast cancer patients.

Introduction

Metastatic breast cancer is a chronic disease requiring specific strategies to control disease progression and related symptoms. Neovascularization, or angiogenesis, is a rate-limiting step during tumorigenesis that promotes tumor cell survival and proliferation by ensuring a supply of oxygen and metabolites. In breast cancer, access to the vasculature also provides means for tissue invasion and metastasis to distant sites [1,2].

Inhibitors of angiogenesis are a new clinical class of drugs with therapeutic potential in oncology [3]. However, defining the most effective dose and schedule for such treatment remains a significant hurdle to their optimal use in the clinic [4]. Thus, the classic maximum tolerable dose (MTD) approach, as defined in the past for cytotoxic drugs, might not be adequate or relevant for this class of drugs which in general have considerably less toxicity than conventional cytotoxics used at MTD, and therefore may be used for prolonged periods to obtain inhibition of new vessel growth, and, in turn, tumor stabilization or shrinkage [5].

Chronic administration of continuous low doses of chemotherapy also referred to as 'metronomic' chemotherapy (MC) has an effect on the breast tumor and other compartments, mainly the vasculature postulating an anticarcinogenic activity; among them, methotrexate, cyclophosphamide, capecitabine, and taxanes are the most studies drugs [6]. The rationale for this approach is preventing effective recovery of the damaged tumor vasculature. This implies that activated tumor vascular endothelial cells may be more sensitive to lower doses of chemotherapeutic drugs compared with normal or cancer cells, when exposed in a frequent or continuous manner [4].

As opposed to maximum tolerated dose (MTD) chemotherapy, main targets of which are presumed to be proliferating tumor cells, the main target of frequent or continuous low dose metronomic chemotherapy are the endothelial cells of growing vasculature of a tumor. In addition, low dose metronomic chemotherapy has very favorable toxicity profile. Omission of prolonged drug-free periods is the key which form basis for anti-angiogenic effects of low dose metronomic chemotherapy regimens, as these breaks are the reasons of repair and recovery from anti-angiogenic effect of chemotherapeutic drugs on developing tumor blood vessels [7].

Capecitabine has proven activity in MBC pretreated with anthracycline and taxane, lower doses (< 1000 mg/m^2 daily) have a more favorable therapeutic index, when compared with standard dosage [8]. Moreover, fixed daily doses and continuous (non-cyclic) dosing schedules have been demonstrated to

be well tolerated and active in MBC [9]. A synergistic effect was observed with the metronomic combination of a fluorouracil prodrug and cyclophosphamides [10].

Given these considerations, we evaluated the efficacy and tolerability of metronomic low-dose capecitabine and oral cyclophosphamide (CTX) combination in metastatic breast cancer in phase II trial and impact of this regimen upon serum vascular endothelium growth factor (VEGF) values when assessed at the baseline and after 2 and 6 months of chemotherapy.

Patients and methods

This prospective phase II study was conducted at Clinical Oncology and Nuclear Medicine Department, Ain Shams University, in the period from June 2006 to March 2008. The study was approved by the Medical Ethics Committee of Faculty of Medicine, Ain Shams University, and written informed consent was obtained from all participants.

Patient eligibility

Eligibility criteria included female patients with metastatic breast cancer. At least one measurable tumor site (target lesion), age > 18 and $\leqslant 75$ years, Eastern Cooperative Oncology Group (ECOG) performance status $\leqslant 2$, adequate bone marrow reserve defined as hemoglobin level $\geqslant 10$ g% white blood cells > 4000 mm^3 and platelets $> 100,000$ mm^3. Adequate renal and liver functions defined as serum creatinine $\leqslant 1.5$ mg/dl, serum bilirubin < 2 mg/dl and aspartate aminotransferase up to three times the upper limit of normal level with life expectancy at least 6 months and signed informed consent. While exclusion criteria included pregnant or lactating patients, presence of cerebral or leptomeningeal metastasis, ascites, pleural effusion or solitary bony lesion as the only sited of disease, any impairment of gastrointestinal function, that may interfere with oral drugs absorption, or serious concomitant systemic disorders incompatible with study design.

Study evaluation and treatment plan

Baseline evaluation included clinical examination, chest X-ray, computed tomography scan (CTS) of abdomen, bone nuclear scan, electrocardiogram (ECG), in addition to complete biochemical and hematological tests. Patients were treated with capecitabine (500 mg) tablet twice daily together with cyclophosphamide (CTX) orally at a dose of 50 mg once daily. Oral antiemetic treatment was allowed. Treatment was given on

out-patients' basis, duration of therapy was based on tumor response, patients with complete response (CR), partial response (PR) or stable disease (SD) could receive treatment until progression, unacceptable toxicity or patient refusal. Cyclophosphamide and capecitabine were reduced by 50% in case of grade $\geqslant 2$ hematologic toxicity, cystitis, GI toxicity, or hand–foot syndrome. To achieve a 50% dose reduction, cyclophosphamide was administrated as one 50-mg tablet every other day and capecitabine was administrated as one 500-mg tablet daily. Serum VEGF was determined at baseline, after 2 and 6 months of treatment.

The change due to follow-up study, delta change (dC) was calculated. It was defined as follows: delta change (dC) = (Post-pre)/Pre or the difference between post and pre as regards pre values for each patient.

Evaluation of VEGF

Quantitative determination of the human VEGF concentration in the serum samples was done by quantitative solid phase ELISA (Quantikine: R&D system, USA). Determination of VEGF concentration was done according to manufacturer's instructions. Each sample was analyzed in triplicate and the mean values were used as the final concentration.

Study assessment

Hematological and biochemical tests were repeated every 28 days. Toxicity was evaluated according to National Cancer Institute Common Toxicity Criteria (NCICT) version 3.0 [12]. Assessment of response was performed according to Response Evaluation Criteria in Solid Tumor (RECIST) [13] after and every 8 weeks of therapy, with appropriate test for each patient.

CR was defined as the disappearance of all known lesions on two separate measurements at least 4 weeks apart, PR was defined as $\geqslant 30\%$ reduction in the sum of the products of the perpendicular diameter of measurable bidimensional lesion, PD was defined as 20% increase with no CR, PR or SD documented before increased disease and SD was defined as neither PR nor PD criteria met.

The disease control rate was defined as the proportion of patients who achieved CRs, PRs or SD.

Study end points

The primary end point of the study was to assess the disease control rate and was related with percentage reduction in VEGF. Secondary objectives were to assess toxicity profile of this regimen, time to disease progression and overall survival.

Statistical analysis

SPSS statistical software package (V. 17, Chicago, IL) was used for data analysis. The following tests were done:

(1) Fridmen's test for comparison between numerical variables with repeated measures and if significant, Wilcoxon signed rank test was applied for pair wise comparison of non-parametric data with corrections of P-values.
(2) Wilcoxon signed rank test for pair wise comparison of non-parametric data with correlation of p-value.

(3) Chi-square test to study the association between the categorical variables data.

Time to progression (TTP) and overall survival (OS) were measured from the date of treatment start to the date of progression and the date of last follow-up or death, respectively, and were assessed using the Kaplan–Meier product limit estimate method. P-value less than 0.05 was considered significant. All tests were two sided.

Results

Patients characteristics

From June 2006 to March 2008, 60 eligible patients were enrolled into the study, the patient characteristics are shown in Table 1. Their age ranged from 47 to 72 years, with a median age of 61 years, the majority was post-menopause (87%). Performance status score 2 was reported in 26 patients (43.3%), positive hormonal status (ER or PR) was recorded in 32 patients (53.3%), while HER-2/Neu was negative in 44 patients (73.3%). One metastatic site was reported in 24 (40%) patients, two metastatic sites were reported in 28 (46.7) and the remaining have three or more metastatic sites. The domain site of metastasis was bone ($n = 28$), followed by liver ($n = 27$), and lung ($n = 21$). The median number of prior chemotherapies was two; 27% had one, 53% had two, and 20% had three or more prior chemotherapies lines for metastatic setting. All patients had progressive disease at study entry. Fifty-one of patients (85%) previously treated with anthracycline and/or taxane containing regimens.

Treatment activity

One patient attained a CR, and partial response was detected in 12 (20%) with an overall RR (21.7%). Twenty one patients had SD with global disease control rates of 56.6%. Disease progression was documented in 26 cases.

Global disease control rates were significantly observed in post-menopause patients and those with positive hormonal and negative HER-2/Neu status. As well as in patients with lung metastasis as compared to other metastatic sites (Table 1).

After a median follow-up period of 16 months (ranged from 4 to 38 months) the median overall survival for all participants was 16 ± 8.02 months (95%CI 13.06–17.28 months) (Fig. 1), for responders (CR and PR) was 24 ± 10.01 months (95% CI 17.08–28.63 months) and for SDs was 19 ± 8.12 months (95%CI 16.08–18.65 months).

The median TTP was 7 ± 2.59 months (95% CI 6.93–9.93) for whole group (Fig. 2).

VEGF measurements and relation with disease progression

Sixty patients had VEGF serum levels measured at baseline, additional measurements were carried out after 2 months ($n = 60$) and 6 months ($n = 58$), respectively, two patients refused and lost follow-up. The median VEGF level in patients with global disease control was significantly decreased with continuous treatment from 442 pg/ml at baseline to 369 pg/ml at 2 months then to 295 pg/ml at 6 months ($p < 0.001$). Although, the median VEGF level in patients with PD was

Table 1 Therapeutic response according to patient and tumor characteristics in patients with metastatic breast cancer.

Factors ($n = 60$)	Maximum response		X^2, P-value
	(CR, PR, SD) ($n = 34$)	(PD) ($n = 26$)	
Age (range 47–72)			
60 years ($n = 30$)	18 (52.9%)	12 (50%)	0.271, 0.602
>60 years ($n = 30$)	16 (47.1%)	14 (50%)	
Menopausal status			
Pre-menopause ($n = 8$)	2 (5.6%)	6 (23%)	4.172, 0.03
Post-menopause ($n = 52$)	32 (94.4%)	20 (77%)	
ER or PR			
−ve ($n = 28$)	8 (23.5%)	20 (77%)	12.902, <0.001
+ve ($n = 32$)	26 (76.5%)	6 (23%)	
Her2/Neu			
−ve ($n = 44$)	32 (94.4%)	12 (46%)	15.084, <0.001
+ve ($n = 14$)	2 (5.6%)	12 (46%)	
Performance status (PS)[a]			
(O + 1) ($n = 34$)	26 (76.5%)	8 (30.8%)	17.778, <0.001
(O + 2) ($n = 26$)	8 (23.5%)	18 (69.2%)	
Previous chemotherapy			
1 ($n = 16$)	12 (35.3%)	4 (15.4%)	22.5, <0.001
2 ($n = 32$)	22 (64.7%)	10 (53.8%)	
3 ($n = 12$)	0 (0%)	12 (30.8%)	
No. of metastases			
1 ($n = 24$)	20 (58.8%)	4 (16.7%)	17.54, <0.001
2 ($n = 28$)	14 (41.2%)	14 (50%)	
3 ($n = 8$)	0 (0%)	8 (33.3%)	
Metastatic site			
Lung ($n = 12$)	12 (100%)	0 (0%)	_[b]
Liver ($n = 8$)	4 (50%)	4 (50%)	
Lung + Liver ($n = 4$)	0 (0%)	4 (100%)	
Lung + Bone ($n = 6$)	6 (100%)	0 (0%)	
Lung + Soft tissue ($n = 8$)	8 (100%)	0 (0%)	
Liver + Bone ($n = 10$)	2 (20%)	8 (80%)	
Lung + Liver + Bone ($n = 4$)	0 (0%)	4 (100%)	
Liver + Bone + Soft tissue ($n = 4$)	0 (0%)	4 (100%)	

[a] PS, performance status according to ECOG (Eastern Cooperation Oncology group); PR, estrogen receptor; PR, progesterone receptor.
[b] No p values because small number of cases within groups.

also significantly decreased ($p < 0.05$) with treatment from 468 pg/ml at baseline to 445 pg/ml at 2 months then to 406 pg/ml at 6 months, the delta change of VEGF in patients with disease control was significantly higher ($p < 0.001$) than in those with PD patients as evaluated after 2 months (−0.155 and −0.06, respectively) and 6 months of therapy (−0.365 and −0.102, respectively) Table 2.

Response and angiogenic activity

Median VEGF for CR and PRs (13 patients) was decreased with treatment from 456 pg/ml at baseline to 340 pg/ml at 2 months then to 258 pg/ml at 6 months ($p < 0.001$). Similarly, the median VEGF level in SD patients ($n = 21$) decreased with treatment from 426 pg/ml at baseline to 385 pg/ml at 2 months then to 310 pg/ml at 6 months ($p < 0.001$), as shown in Table 3. Moreover, the delta change of VEGF in responding cases was significantly higher than in SD patients either after 2nd (−0.210 and −0.120, respectively) or 6th months of therapy (−0.390 and −0.290, respectively).

Treatment compliance

A total of 257 cycles (months) of therapy were administered with median administration time per patients of 4 months (range; 2–16 months). Only 13% of cycles were delayed and 10% of courses administered at reduced dosages.

The overall regimen was well tolerated. Palmar–plantar erythrodythesia was the most common side effect (Grades 1 and 2 in 36.7% of cases). Leucopenia (Grades 1 and 2) was the most frequent hematological toxicity and was observed in 27 patients. Grade 3 elevation of serum transaminases was reported in 8% of patients necessitating transient cessation of Cap and 50% dose reduction in the subsequent cycles. All the previous toxic effects were reversible. No treatment related mortality or febrile neutropenia was recorded (Table 4).

Discussion

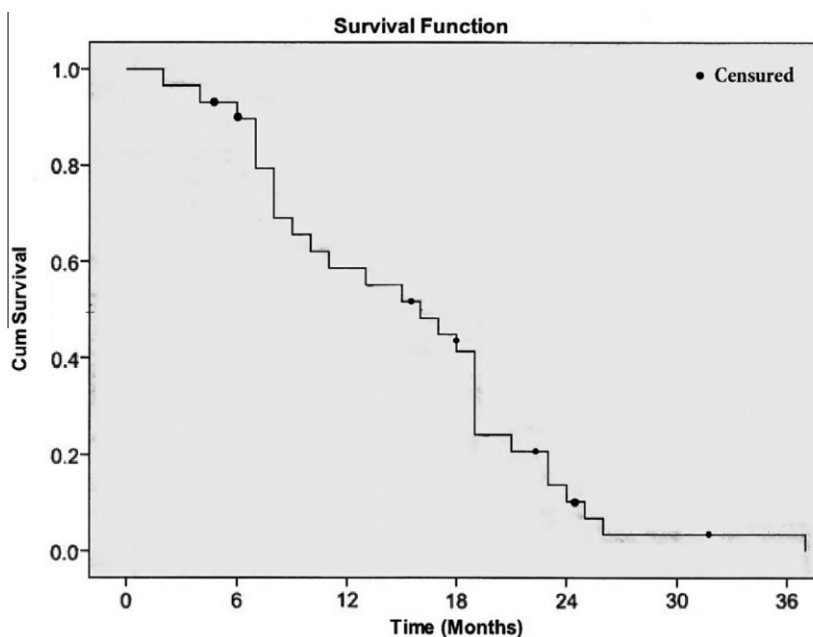Metronomic chemotherapy (MC) regimens have been shown to exert antiangiogenic activity, by blocking the supply of

Figure 1   Overall survival for the studied metastatic breast cancer patients.
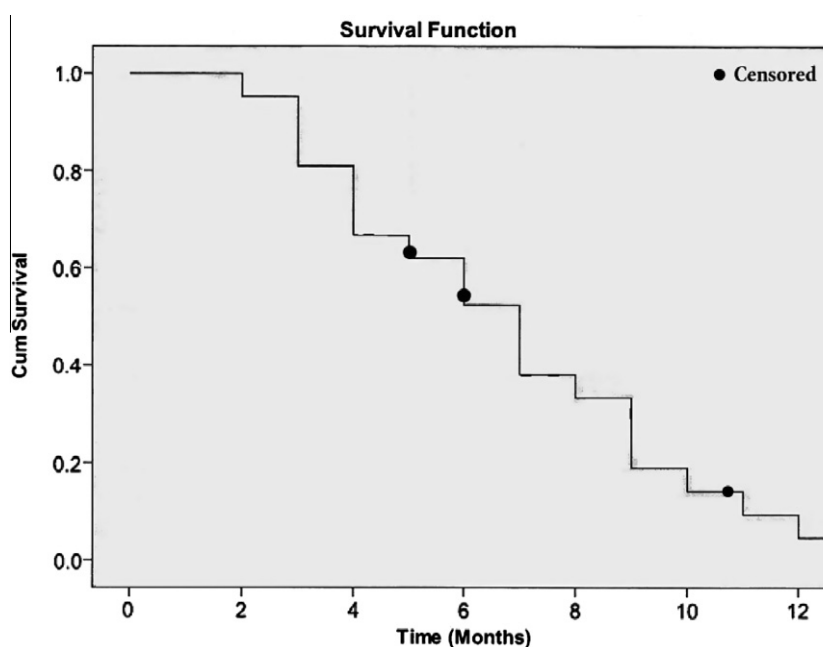

Figure 2   Progression free survival for the studied metastatic breast cancer patients.

essential nutrients and removal of metabolites to malignant cells. So, it delays both primary tumor and metastasis growth [11]. Over the last few years, numerous studies evaluating MC alone or in combination with other antiangiogenic agents in advanced breast [4], prostate [14], and ovarian [15] cancer among other tumor types [2,16,17] have been reported. MC has emerged as an effective treatment with a major clinical advantage: it minimizes the toxic side effects of the drugs employed, thereby allowing their safe and long term administration [18]. In the current study, a new schedule was designed

with metronomic administration of Cap and oral cyclophosphamide. This schedule has shown synergistic antitumor activity without significant toxicity [10].

Unlike studies evaluating the activity of classic cytotoxic agents, where shrinkage in tumor size is the objective, the absence of disease progression assumes a great importance as an end point in clinical trials using MC. Accordingly, our results revealed that more than half of the patients included in the trial are likely to benefit from this treatment (CR, PR + SD = 57%). It is also encouraging that the results we

Table 2 Serum VEGF concentrations (pg/ml) at baseline, 2 months and 6 months after treatment according to disease control in patients with metastatic breast cancer.

VEGF (pg/ml)	Disease response		P-value
	Non-progressive (PR, CR, SD) median (range)	Progressive disease (PD) median (range)	
Base line	442 (437.92–461.31) ($n = 34$)	468 (461.58–489.26) ($n = 26$)	
2 months	369 (326.17–372.05) ($n = 34$)	445 (432.65–452.63) ($n = 26$)	
6 months	295 (247.26–301.07) ($n = 34$)	406 (380.35–420.25) ($n = 24$)	
Signed-rank test of VEGF between base line and 2 months	$P < 0.001$	$P < 0.001$	
Signed-rank test of VEGF base line and 6 months	< 0.001	$P < 0.001$	
Delta change of VEGF between base line and 2 months	0.155 (−0.1659 to 0.2719)	0.060 (0.0488–0.0639)	< 0.001
Delta change of VEGF between base line and 6 months	0.365 (−0.3206 to 0.4494)	0.102 (0.2021–0.1150)	< 0.001
Signed-rank test between 2 months and 6 months	$P < 0.0001$	$P = 0.0001$	

Table 3 Serum VEGF concentrations (pg/ml) at baseline, 2 months and 6 months after treatment according to therapeutic response in patients with metastatic breast cancer.

VEGF (pg/ml)	Therapeutic response		P-value
	Complete and partial response (CR, PR) median (range)	Stable disease (SD) median (range)	
Base line	456 (435.5–458.8)	426 (432.7–469.6)	
2 months	340 (272–351) (PR = 13)	385 (347.6–398.3) (SD = 21)	
6 months	258 (177–265.4) (CR = 1, PR = 12)	310 (280–335.5) (SD = 21)	
Signed-rank test of VEGF base line and 2 months	$Z = -3.3$, $P = 0.001$	$Z = -4.11$, $P < 0.001$	
Signed-rank test of VEGF base line and 6 months	$Z = -3.3$, $P = 0.001$	$Z = -4.11$, $P < 0.001$	
Delta change of VEGF between base line and 2 months	0.210 (0.3930–0.2041)	0.120 (−0.2267 to 0.1097)	$Z = -3.184$, $P = 0.001$ (HS)
Delta change of VEGF between base line and 6 months	0.390 (0.6042–0.4043)	0.290 (−0.3809 to 0.2373)	$Z = -2.794$, $P = 0.005$ (HS)

Table 4 Toxicity of the treatment in patients with metastatic breast cancer.

Toxicity	Grade I No. (%)	Grade II No. (%)	Grade III No. (%)	Total No. (%)
Leucopenia	16 (27%)	2 (3.3%)	1 (1.7%)	19 (32%)
Neutropenia	7 (12%)	3 (5%)	–	10 (17%)
Thrombocytopenia	2 (3.3)	–	–	2 (3.3)
Anemia	13 (17%)	3 (5%)	–	16 (26.7%)
Nausea and vomiting	12 (20%)	5 (8%)	–	17 (28.3%)
Diarrhea	9 (15%)	3 (5%)	–	12 (20%)
Hand and foot syndrome	19 (32%)	3 (5%)	–	22 (36.7%)
Mucositis	7 (12%)	1 (1.7)	–	8 (13.3%)
↑ Transaminases	9 (15%)	3 (5%)	5 (8%)	17 (28.3%)
Alopecia	1 (1.7%)	–	–	1 (1.7%)

have obtained using MC showed significant reduction of the median VEGF level among the non-progressed group from 442 at the base line to 369 after two months then 295 after 6 months, signifying the usefulness of this regimen. These results confirm previous studies [18,19].

Another important aspect of our results relates to the response rate with chronic low-dose treatment of the chemotherapeutic drugs that we studied. As reported in Table 3, the median VEGF level for patients with CR or PR was declined from 456 at the base line to 340 after 2 months the 258 after 6 months (at $P = 0.001$). Similarly those with SD their median VEGF level were significantly reduced, which emphasizes the great response of the patients included in the trial and advantage of using MC in reducing the VEGF level. 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 have indicated that values of VEGF can be reduced after treatment with agents inducing an antiangiogenic activity [19–21], and that VEGF can be considered as a marker of the regulation of angiogenic factors [22].

Our results strengthen the conclusion that the antitumor effects of low-dose metronomic chemotherapy are attributable, at least in part, to a mechanism involving inhibition of tumor blood vessel formation. In addition to antiangiogenic mechanisms in which fully differentiated endothelial cells are growth-inhibited and/or killed by metronomic low-dose chemotherapy, an antivasculogenic process may also be involved that is mediated through effects on reducing circulating endothelial progenitors (CEP) mobilization and viability [23].

The palliative goal of treatment in MBC and the achievement of symptomatic control and maintenance of quality of life are desirable treatment end points. Accordingly, MC has revealed several new and important aspects. These include the success of the treatment strategy on blocking angiogenesis (effect of CTX) and improving therapeutic index (Cap) [8]. Importantly, this regimen lacks major toxicities (bone marrow suppression mucositis or hair loss). Thus, it may represent the paradigm of effective/low-toxic anticancer therapy [10,24].

The identification of patients who might benefit from MC is crucial for optimization of the treatment strategy. Postmenopausal status, expression of steroid hormone receptors; lung metastasis as well as single site of disease were significantly observed more in patients who achieved clinical benefit. These results are in line with previously reported data [18,19]. In our series, the recorded lower efficacy of MC in heavily pretreated patients might be related to redundancy of angiogenic factors

vascular remodeling related to previous exposure to chemotherapy, angiogenesis-independent tumor growth, increased hypoxia tolerance all lead to acquire resistance [25].

Combination of anti-angiogenic drugs as bevacizumab with MC induce remarkable responses with sustained tumor regression in advanced breast cancer [15,22]. But for economic consideration it could not be used on regular basis in MBC patients.

In conclusion, low-dose, oral CTX, and Xeloda demonstrated significant efficacy in metastatic breast cancer and provided disease control for a significant proportion of patients. Increased attention to patients' quality of life favors the use of an active oral treatment. The low burden of personal costs (subjective toxicity and infrequent visits to care providers) and the possibility to continue the treatment for several months in responders (as often required for patients with advanced breast cancer who respond positively to chemotherapy). VEGF can be considered as predictive marker of response to MC. Although such preliminary results are encouraging, they need to be confirmed in much larger, controlled and prospective randomized clinical trials to substantiate the survival benefit of the less toxic low-dose/continuous chemotherapy protocols over the standard MTD protocols.

References

[1] MacKenzie KL. Lock RB: an endothelial metronome breast cancer? Blood 2006;108:407–8.

[2] Tuettenberg J, Grobholz R, Marc MZ, Brockmann A, Lohr F, Wenz F, et al. Recurrence pattern in glioblastoma multiforme patients treated with anti-angiogenic chemotherapy. J Cancer Res Clin Oncol 2009;47:835–45.

[3] Ellis LM, Eckhardt SG. Integrating molecular oncology into clinical practice: antiangiogenic therapy. J Clin Oncol 2005;23:937–8.

[4] Ademuyiwa FO, Miller KD. Incorporation of antiangiogenic therapies in the treatment of metastatic breast cancer. Clin Breast Cancer 2008;8(4):S151–6.

[5] Mancuso P, Colleoni M, Calleri A, Orlando L, Maisonneuve P, Pruneri G, et al. Circulating endothelial cell kinetics and viability predict survival in breast cancer patients receiving metronomic chemotherapy. Blood 2006;108(2):452–9.

[6] Sarmiento R, Gasparini G. Antiangiogenic metronomic chemotherapy. Onkologie 2008;31:161–2.

[7] Hirata S, Matsubara T, Saura R, Hirohata K, Tateishi H. Inhibition of in vitro vascular endothelial cell proliferation and in vivo revascularization by low dose methotrexate. Arthritis Rheum 1989;32:1065–73.

[8] Nannini M, Bobili E, Di Cicilia R, Brandi G, Maleddu A, Pantaleo MA, et al. To widen the setting of cancer patients who could benefit from metronomic capecitabine. Cancer Chemother Pharmacol 2009;64(1):189–93.

[9] Hennessy BT, Gauthier AM, Michaud LB, Hortobagyi G, Valero V. Lower dose capecitabine has a more favorable therapeutic index in metastatic breast cancer: retrospective analysis of patients treated at MD Anderson cancer center and a review of capecitabine toxicity in the literature. Ann Oncol 2005;16:1289–96.

[10] Dellapasqua S, Bertolini F, Bagnardi V, Campagnoli E, Scarano E, Torisi R, et al. Metronomic cyclophosphamide and capecitabine combined with Bevacizumab in advanced breast cancer. J Clin Oncol 2008;26(3):4899–905.

[11] Orlando L, Cardillo A, Rocca A, Balduzzi A, Ghisini R, Peruzzotti G, et al. Prolonged clinical benefit with metronomic chemotherapy in patients with metastatic breast cancer. Anticancer Drugs 2006;17:961–7 [Copyright© Lippincott Williams and Wilkins].

[12] Casuati D. Toxicities of chemotherapy NCI common toxicity criteria version 3. In: Casciato D, editor. Manual of clinical oncology, fifth ed., vol. 698. Philadelphia: Lippincott Williams and Wilkins; 2004. p. 703.

[13] Therasse P, Arbuch SG, Eisenhauer EA. New guidelines to evaluate the response to treatment in solid tumors: European organization for research and treatment of cancer. Natl Cancer Inst 2000;92:205–16.

[14] Lord R, Nair S, Schache A, Spicer J, Somaihah N, Khoo V, et al. Low dose metronomic oral cyclophosphamide for hormone resistant prostate cancer: a phase II study. J Urol 2007;177:2136–40.

[15] Garcia AA, Hirte H, Fleming G, Yang D, Tsao-Wei DD, Roman L, et al. Phase II clinical trial of bevacizumab and low-dose metronomic oral cyclophosphamide in recurrent ovarian cancer: a trial of the California, Chicago, and Princess Margaret Hospital phase II consortia. J Clin Oncol 2008;26:76–82.

[16] Gille J, Spieth K, Kaufmann R. Metronomic low-dose chemotherapy as antiangiogenic therapeutic strategy for cancer. J Dtsch Dermatol Ges 2005;3:26–32.

[17] Calvani N, Orlando L, Nacci A, Sponziello F, Cinefra M, Cinier S. Metronomic chemotherapy against cancer: from paradigm to clinical practice? Tumori 2009;95:843–5.

[18] Colleoni M, Orlando L, Sanna G, Rocca A, Maisonneuve B, Peruzzotti G, et al. Metronomic low-dose oral cyclophosphamide and methotrexate plus or minus thalidomide in metastatic breast cancer: antitumor activity and biological effects. Ann Oncol 2006;17:232–8.

[19] Fersis N, Samyczek-Gargyo B, Armenau S, Gagulic E, Pantic L, Relakis K, et al. Changes in vascular endothelial growth factor VEGF after chemoendocrine therapy in breast cancer. Eur J Gynaecol Oncol 2004;25:45–50.

[20] Stoll BR, Migliorini C, Kadambi A, Munn LL, Jain RK. A mathematical model of the contribution of endothelial progenitor cells to angiogenesis in tumors: implications for anti-angiogenic therapy. Blood 2003;102:2555–61.

[21] Emmenegger U, Morton GC, Francia G, Shaked Y, Franco M, Weinerman A, et al. Low dose metronomic daily cyclophosphamide and weekly tirapazamine: a well-tolerated combination regimen with enhanced efficacy that exploits tumor hypoxia. Cancer Res 2006;66:1664–74.

[22] Shah Ma, Ramanathan RK, Ilson DH, Levnor A, D'Adamo D, O'Reilly E. Multicenter phase II study of irinotecan, cisplatin, and bevacizumab in patients with metastatic gastric or gastroesophageal junction adenocarcinoma. J Clin Oncol 2006;24(33):5201–6.

[23] Bertolini F, Paul S, Mancuso P, Monestiroli S, Gobbi A, Shaked Y, et al. Maximum tolerable dose and low-dose metronomic chemotherapy have opposite effects on the mobilization and viability of circulating endothelial progenitor cells. Cancer Res. 2003;63:4342–6.

[24] Daenen LG, Shaked Y, Man S, Xu P, Voest EV, Hoffman RM, et al. Low-dose metronomic cyclophosphamide combined with vascular disrupting therapy induces potent antitumor activity in preclinical human tumor xenograft model. Mol Cancer Ther 2009;8(10):2872–81.

[25] Kerbel RS, Yu J, Tran J, Man S, Viloria Petit A, Klement G, et al. Possible mechanism of acquired resistance to anti-angiogenic drugs: implication for the use of combination therapy approach. Cancer Metastasis Rev 2001;20:79–86.