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Metronomic low-dose oral cyclophosphamide and methotrexate plus or minus thalidomide in metastatic breast cancer: antitumor activity and biological effects

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Background: We previously demonstrated efficacy and impact on serum vascular endothelial growth factor (VEGF) for metronomic cyclophosphamide (C) and methotrexate (M) in patients with breast cancer. New metronomic schedules were investigated.

Patients and methods: Patients with advanced breast cancer were randomized to receive oral C (50 mg daily) and M (2.5 mg twice daily on days 1 and 4) (arm A) or the same regimen plus thalidomide (200 mg daily) (arm B).

Results: The mean VEGF level decreased from 378.9 (± 274.4) pg/ml at baseline to 305.9 (± 203.6) pg/ml at 2 months ($P < 0.001$), with similar change with respect to baseline in both arms. In 171 evaluable patients we observed three complete remissions (CR) in both arms A and B, 15 partial remission (PR) in arm A and seven in arm B, for an overall response of 20.9% [95% confidence interval (CI) 12.9% to 31%] in arm A and 11.8% (95% CI 5.8% to 20.6%) in arm B. The clinical benefit (CR + PR + SD ≥ 24 weeks) was 41.5% for both arms. Toxicity was generally mild. Higher neurological toxicity (2% versus 60%; $P < 0.0001$) and constipation (8% versus 51%; $P < 0.0001$) was observed in arm B.

Conclusions: Metronomic low-dose CM induced a drop in VEGF, and was effective and minimally toxic. The addition of thalidomide did not improve results.

Key words: angiogenesis, breast cancer, cyclophosphamide, methotrexate, metronomic chemotherapy

introduction

Cytotoxics typically lead directly or indirectly to DNA damage and disrupt DNA replication in proliferating cells. Usually, chemotherapy is designed to kill as many tumor cells as possible by treating with maximum tolerated doses (MTDs), allowing normal tissues to recover. In general, responses of overt disease are of short duration, with tumor growth, which occurs despite the administration of the same drug regimen.

Results from animal models suggest that chronic administration of low doses of chemotherapy has an effect on the tumor and other compartments, mainly the vasculature [1, 2]. Chronically administered cyclophosphamide (C) at a low dose produces apoptosis of endothelial cells in the tumor microvasculature with a compromised repairing process, therefore inducing a prolonged antiangiogenic effect of the drug [3]. It was also reported that mouse tumors resistant to a conventionally administered drug (MTD schedule) might respond for a long period of time to the same drug when a lower, more frequent dose scheduling was used [1]. The benefit of the 'chronic' low-dose administration of drug was attributed to activity directed to the drug-sensitive endothelial cell compartment of the tumors [4]. Clinical studies support the notion, that non-toxic, low-dose 'metronomic' chemotherapy may be useful. We demonstrated that low-dose C and methotrexate (M) can induce tumor regression in 19% of patients with advanced breast cancer, with an overall clinical benefit of 31.7% [complete remission (CR) + partial remission (PR) + stable disease (SD) ≥ 24 weeks] and causing only minimal toxicity [5].

In animal models the efficacy of continuous low-dose, metronomic chemotherapy can be enhanced by their combination with other antiangiogenic, endothelial-specific drugs [6, 7]. Thalidomide, a derivative of glutamic acid, has immune-modulating activity secondary to inhibition of lymphocyte proliferation [8]. The drug also inhibits tissue tumor necrosis factor-α production by stimulated human monocytes and lymphocytes [9, 10]. In addition to its immune-modulatory activities, oral thalidomide inhibits

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angiogenesis induced by basic fibroblast growth factor and vascular endothelial growth factor (VEGF) in the rabbit corneal micropocket assay [11, 12].

In a phase II study in patients with progressive metastatic breast cancer randomized to receive either daily 200 mg of thalidomide or 800 mg, modest activity was registered with two patients on the 200 mg dose level that had SD at 8 weeks. Thalidomide was well tolerated at the 200 mg dose level. In contrast, the 800-mg dose level was not as well tolerated, with dose reductions required in 50% of the patients [13]. Biological activity was observed with thalidomide given at very low doses (100 mg daily) in advanced prostate cancer with concurrent changes in serum growth factor levels and prostate-specific antigen (PSA). In particular, in responding patients concurrent PSA decline and decrease in mean values of VEGF was registered, supporting the use of thalidomide at low dose levels [14].

Previous studies showed that thalidomide potentiates the antitumour activity of C against murine tumors. In particular, pharmacokinetic interaction was shown, with thalidomide extending the half-life of cyclophoshamide [15]. Moreover, C, M and thalidomide have different mechanisms of antitumor activity and non-overlapping side-effect profiles, and are individually well tolerated supporting their combination.

We therefore evaluated the activity and biological effects of a new schedule of low-dose oral C and M and compared the biological effect of this combination with the same combination plus thalidomide.

patients and methods

patients selection

Patients included in the study were required to have histologically confirmed metastatic breast carcinoma either pretreated or not after a previous line of chemotherapy for metastatic disease. The inclusion of patients untreated with chemotherapy for metastatic disease was allowed by the ethics committee based on the promising activity results shown in this subgroup of patients in a previously published study [5]. Other inclusion criteria were measurable or evaluable disease, age ≤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, and adequate renal (serum creatinine <120 μmol/l) and hepatic (serum bilirubin <20 μmol/l, aspartate aminotransferase <60 IU/l) function. Each patient included in the study gave a written informed consent. The protocol was reviewed and approved by institutional review boards.

study evaluation and treatment

Baseline evaluation included clinical examination, chest X-ray, liver ultrasound or computed tomography scan, bone nuclear scan, ECG, complete biochemical and hematological tests. Complete blood count was then repeated every 14 days and biochemical tests every 28 days.

Randomization was conducted at the European Institute of Oncology after stratification according to pretreatment. Patients were randomized to M orally at a dose of 2.5 mg twice a day on days 1 and 4 every week (10 a.m., 5 p.m.) and C orally at a dose of 50 mg a day (9 a.m.) (arm A) or the same treatment plus thalidomide administered orally at the dose of 200 mg/day (9 p.m.). No antiemetic treatment was prescribed. Serum VEGF was determined at baseline, after 2 and 6 months of treatment, and when progressive disease was diagnosed as previously described [5].

side-effects and response

Toxicity was evaluated according to National Cancer Institute Common Toxicity Criteria criteria by clinical and laboratory investigations. Treatment was withheld and delayed for 1 week in case of a neutrophil count <1000 mm^3 and/or platelet count <75 000 mm^3. A 50% dose reduction in the total amount of drug administered in each cycle was prescribed after hematological recovery. In case of a neutrophil count <1500 but >1000 mm^3 and/or platelet count <100 000 but >75 000 mm^3, therapy was administered with a 50% dose reduction in the total amount of drug administered in each cycle. Re-escalation of drug doses was attempted if close monitoring was possible.

In the event of grade ≥2 anorexia, nausea, vomiting, diarrhea, stomatitis, dryness of the mouth, epigastric pain or increase in transaminases, all therapy was postponed until symptoms subsided. A 50% reduction of CM was performed for the next cycle, with subsequent re-escalation to full dosage if tolerated. Any other non-hematological grade 3 toxicity was managed by a 50% reduction of dosage in the next cycle, which was not commenced until full recovery had occurred.

Thalidomide was withheld for grade 2 neurotoxicities, except drowsiness and somnolence, until resolution to grade ≤1, and then restarted at a 50% dose reduction of the original dose. For recurrent grade 2 neurotoxicity, thalidomide was withheld. If the patient developed intolerable drowsiness or somnolence at the starting dose of 200 mg/day, the dose was reduced to 100 mg/day. If the patient could not tolerate 100 mg/day, thalidomide was withheld.

Assessment of response was performed according to WHO criteria after every 8 weeks of therapy. The clinical benefit rate was defined as the proportion of patients who achieved CRs, PRs or SD for at least 24 weeks.

statistical analysis

The main end point of the study was percentage reduction in VEGF after 2 months of chemotherapy. Previous experiments with similar cytotoxic agents yielded a mean decrease of 20% with a standard deviation of 38% [5]. It was anticipated that the thalidomide arm would be associated with a greater mean percentage decrease of VEGF of 20%. Thus the study was designed to detect a difference in the mean percentage decrease of VEGF of 20% in the standard therapy arm compared with 40% in the thalidomide arm. A sample size of 80 patients per arm would provide a 90% power to detect the difference in mean percentage reduction in VEGF of 20%.

Secondary end points were progression-free survival, defined as the length of time from the date of treatment to the date of progression, and overall survival, defined as the time from treatment until the date of death (from any cause) or the date of last follow-up. The response duration was measured from the date of achievement of response. Fisher's exact test and the Mantel–Haenszel χ^2-test for trend were used to assess the association between categorical and ordinal variables and treatment. Survival plots were drawn using the Kaplan–Meier method. The log-rank test was used to assess the survival difference between strata.

results

One hundred and seventy-eight patients were randomized into the trial between June 2000 and November 2003. Main patient characteristics are shown in Table 1. Fifteen (8.4%) patients were aged 70 years or older. In the subgroup of untreated patients, 44 (63%) and 22 (31%) had respectively one and two sites of disease.

One hundred and forty-one patients had VEGF serum levels measured at baseline and 115 had an additional

Table 1. Characteristics of eligible patients according to treatment arm

Strata	No. patients	Arm A		Arm B		*P* value
		n	%	*n*	%	
Entered/eligible	178	90	50.6	88	49.4	
Median age, years (range)		53.8 (33–77)		54.6 (31–78)		0.22
Progressive disease at study entry						
No	55	29	52.7	26	47.3	
Yes	123	61	49.6	62	50.4	0.75
Baseline ECOG performance status						
0	150	78	52.0	72	48.0	
1	28	12	42.9	16	57.1	0.42
Pretreatment (CT)						
No	70	37	52.9	33	47.1	
Yes	108	53	49.1	55	50.9	0.65
No. of sites						
1	89	43	48.3	46	51.7	
2	63	31	49.2	32	50.8	
3+	26	16	61.5	10	38.5	0.31
Tumor sites						
Lung	38	20	52.6	18	47.4	0.86
Liver	76	40	52.6	36	47.4	0.65
Bone	82	38	46.3	44	53.7	0.37
Other sites	84	48	57.1	36	42.9	0.11
ER						
Absent	63	32	50.8	31	49.2	
Present	99	50	50.5	49	49.5	1.00
PgR						
Absent	79	45	57.0	34	43.0	
Present	78	35	44.9	43	55.1	0.15
Ki67						
<20%	56	33	58.9	23	41.1	
≥20%	26	12	46.2	14	53.8	0.34
Her-2/Neu						
0/+/++	83	45	54.2	38	45.8	
+++	19	11	57.9	8	42.1	0.80
pT[a]						
pT1	44	19	43.2	25	56.8	
pT2	50	22	44.0	28	56.0	
pT3–4	10	7	70.0	3	30.0	0.26
pN[b]						
pN0	31	13	41.9	18	58.1	
pN1-pN2	70	35	50.0	35	50.0	0.52
Chemotherapy for advanced disease						
None	70	37	52.9	33	47.1	
1 line	62	27	43.5	35	56.5	
2 or more lines	46	26	56.5	20	43.5	0.91
Endocrine therapy for advanced disease						
None	61	33	54.1	28	45.9	
1 line	52	27	51.9	25	48.1	
2 or more lines	65	30	46.2	35	53.8	0.37

ECOG, Eastern Cooperative Oncology Group; CT, chemotherapy; ER, estrogen receptor; PgR, progesterone receptor; a, b at previous surgery.

measurement after 2 months. Two months after treatment, VEGF level was significantly decreased, with similar change with respect to baseline, for both untreated and pretreated patients, in arms A and B ($P = 0.94$) (Table 2). Overall, the mean VEGF level decreased with treatment from 378.9 ± 274.4 pg/ml at baseline to 305.9 ± 203.6 pg/ml at 2 months ($P < 0.001$, Wilcoxon matched pairs test), with a mean reduction of 73.1 pg/ml (95% confidence interval (CI) 41.1–105]. For both treatment groups combined, there was a 30% reduction in serum VEGF after 2 months in patients with complete or partial therapeutic response ($P < 0.0001$), a 14% reduction in patients with SD ($P = 0.006$), but no significant

reduction (8%; $P = 0.82$) in patients with progressive disease (Table 3).

Of 171 evaluable patients for response (arm A, 86 patients; arm B, 85 patients; seven not evaluable due to treatment stopped within the first month: one early death, three patient's preference, three due to toxicity) there were three CRs in each of the treatment arms, 15 PRs in arm A and seven in arm B, for an overall response of 20.9% (95% CI 12.9% to 31%) in arm A and 11.8% (95% CI 5.8% to 20.6%) in arm B ($P = 0.15$). Additional long-term disease stabilization (SD after 24 weeks) was seen in 18 patients in arm A and 25 in arm B. The overall clinical benefit (CR + PR + SD ≥24 weeks) was 41.5% (95% CI 34% to 49.3%) for both arms.

In untreated patients we observed three CRs in arm A and one in arm B, 10 PRs in arm A and 2 in arm B, for an overall response of 36.1% (95% CI 20.8% to 53.8%) in arm A and 9.1% (95% CI 1.9% to 24.3%) in arm B ($P = 0.01$) and a global clinical benefit of 55.6% (95% CI 38.1% to 72.1%) in arm A and 39.4% (95% CI 22.9% to 57.9%) in arm B ($P = 0.23$). In pretreated patients we observed no CRs in arm A and two in arm B, five PRs in arm A and five in arm B, for an overall response of 10% (95% CI 3.3% to 21.8%) in arm A and 13.5% (95% CI 5.6% to 25.8%) in arm B ($P = 0.76$) and a global clinical benefit of 32% (95% CI 19.5% to 46.7%) in arm A and 42.3% (95% CI 28.7% to 56.8%) in arm B ($P = 0.31$).

The median treatment duration was similar for arm A (4 months; range 1–25) and arm B (4 months; range 1–27). Median time to response was 63 and 62 days ($P = 0.85$), median time to progression 3.8 and 4.1 months ($P = 0.46$) and overall survival 18.2 and 17.1 months ($P = 0.98$) in arms A and B, respectively. In untreated patients a median time to progression of 5.7 and 4 months ($P = 0.37$) and an overall survival of 26.2 and 28.4 months ($P = 0.68$) were observed for arms A and B, respectively. The clinical benefit observed was significantly lower for patients with liver metastases if compared with other sites (31.4 versus 48.5%; $P = 0.028$) (Table 4).

Of all the clinical characteristics of the patients, only the presence of liver metastasis was significantly associated with worse progression-free survival [hazard ratio (HR) 1.38; 95% CI 1.01–1.88; log-rank $P = 0.04$] and overall survival (HR 2.01; 95% CI 1.37–3.24; log-rank $P = 0.0005$).

Table 5 summarizes the side-effects observed. Treatment was well tolerated. The most frequently encountered toxicity was increased values of transaminases, which was observed in 56% of cases. A complete recovery was achieved in all cases after reduction or transient interruption of M.

Thalidomide was correlated with a significantly higher incidence of grade 2 neutropenia, grade 1–2 stipsis and grade 1–2 neuropathy (Table 5). Moreover, two patients treated with thalidomide presented deep venous thrombosis. A significantly lower incidence of grade 1 liver toxicity (transaminase elevation) was observed with CM and

Table 2. Serum vascular endothelial growth factor concentrations (pg/ml) at baseline and 2 months after treatment between the two study arms[a]

	n	Mean	95% CI	*P* value
All patients at baseline				
Only C + M	74	384.7	321.8 to 447.5	
C + M + thalidomide	67	388.3	321.5 to 455.1	
Difference		−3.6	−94.5 to 87.2	0.94
All patients at 2 months				
Only C + M	59	302.4	251.3 to 353.5	
C + M + thalidomide	56	303.3	246.5 to 360.1	
Difference		0.9	−73.9 to 75.8	0.98
All patients, change				
Only C + M	58	−71.9	−119.7 to −24.0	
C + M + thalidomide	55	−74.3	−117.8 to −30.9	
Difference		2.5	−61.7 to 66.6	0.94
Untreated patients at baseline				
Only C + M	32	377.6	299.2 to 456.0	
C + M + thalidomide	26	409.8	280.6 to 538.9	
Difference		−32.2	−173.9 to 109.6	0.66
Untreated patients at 2 months				
Only C + M	27	321.5	249.2 to 393.8	
C + M + thalidomide	20	329.1	230.1 to 428.0	
Difference		−7.6	−123.7 to 108.5	0.90
Untreated patients, change				
Only C + M	27	−45.8	−95.5 to 4.0	
C + M + thalidomide	20	−96.8	−187.8 to −5.8	
Difference		51.0	−43.1 to 145.2	0.31
Pretreated patients at baseline				
Only C + M	42	390.1	293.9 to 486.2	
C + M + thalidomide	41	374.7	297.9 to 451.5	
Difference		15.4	−106.2 to 136.9	0.80
Pretreated patients at 2 months				
Only C + M	32	286.3	211.3 to 361.4	
C + M + thalidomide	36	288.9	216.5 to 361.4	
Difference		−2.6	−105.1 to 99.9	0.96
Pretreated patients, change				
Only C + M	31	−94.6	−175.0 to −14.2	
C+ M + thalidomide	35	−61.5	−109.3 to −13.6	
Difference		−33.1	−122.4 to 56.2	0.47

[a]Two months after treatment, vascular endothelial growth factor level had decreased significantly, with equal change between the two arms.
CI, confidence interval; C, cyclophosphamide; M, methotrexate.

Table 3. Serum VEGF concentrations (pg/ml) at baseline and 2 months after treatment according to therapeutic response

VEGF	Therapeutic response at 2 months		
	Response within 2 months ($n = 47$)	Stable disease at 2 months ($n = 32$)	Progression within 2 months ($n = 34$)
Baseline	412 ± 305	346 ± 207	363 ± 286
2 months	290 ± 195	299 ± 185	334 ± 232
Difference	−123 ± 163 (30% reduction)	−47 ± 107 (14% reduction)	−29 ± 214 (8% reduction)
Signed-rank test	$P < 0.0001$	$P = 0.006$	$P = 0.82$

VEGF, vascular endothelial growth factor.

thalidomide, suggesting a protective effect of thalidomide on hepatic toxicity due to M, as previously reported by others [16].

Only three patients in arm A (3%) and three in arm B (3%) discontinued C and M. Thalidomide was discontinued in 14 patients (16%) in arm B. The dose of M was reduced in seven patients (8%) in arm A and three patients (3%) in arm B, and the dose of thalidomide was reduced in seven patients (8% in arm B). Reasons for either discontinued or reduced metronomic chemotherapy (CM) were mainly leukopenia and increase in transaminases, while thalidomide was mainly stopped due to neurologic toxicity.

Table 4. Clinical benefit according to site of metastases and biological features

	Progressive disease	Response or stable disease >24 weeks [*n* (%)]	*P* value
No. of metastases			
1	49	38 (44)	
2+	51	33 (39)	0.64
Lung metastases			
No	78	56 (42)	
Yes	22	15 (41)	1.00
Liver metastases			
No	52	49 (49)	
Yes	48	22 (31)	0.028
Bone metastases			
No	57	37 (39)	
Yes	43	34 (44)	0.54
Other metastases			
No	52	37 (42)	
Yes	48	34 (41)	1.00
ER and PgR			
Both absent	33	20 (38)	
Other	62	40 (39)	1.00
Her2/Neu			
0/+/++	47	32 (41)	
+++	11	7 (39)	1.00

ER, estrogen receptor; PgR, progesterone receptor.

discussion

The concept of metronomicly delivered therapy has become relevant for the treatment of cancer. Recent publications on *in vitro* activity of taxanes and vinca alkaloids at chronic, low-dose exposure, which resulted in inhibiting vessel formation and growth [17, 18], support the concept that the more frequent pace of administration is important for conferring efficacy to this schedule of chemotherapy. Fluorouracil administered continuously at low doses, which was reported to be successful for treating patients with breast carcinoma, appears to be as the first example of a metronomic schedule [19]. Also, weekly paclitaxel appeared to be more active than standard 3-weekly administration of the drug in the preoperative setting [20]. Metronomic, low-dose chemotherapy alone or in combination with proapoptotic biomodulators has demonstrated activity in patients with hormone-refractory prostate carcinoma [21], heavily pretreated sarcomas [22] and melanomas [23].

The current trial, the largest clinical experience of low-dose continuous chemotherapy for patients with advanced breast cancer, confirms a role for metronomic chemotherapy. A new schedule was designed in order to better distribute M during the treatment period (every 4 days, rather than for two consecutive days every week) in an attempt to reduce hepatic toxicity. C was given every day, as in the past series. This schedule had gained some confirmation of experimental efficacy

Table 5. Side-effects

Side effect	Grade 1			Grade 2			Grade 3–4		
	C+M	C+M+T	*P* value	C+M	C+M+T	*P* value	C+M	C+M+T	*P* value
Anaemia	10 (11%)	18 (21%)	0.15	2 (2%)	2 (2%)	1.00	2 (2%)	1 (1%)	1.00
Leukopenia	23 (26%)	17 (20%)	0.57	16 (18%)	21 (24%)	0.56	3 (3%)	5 (6%)	0.71
Neutropenia	17 (20%)	13 (15%)	0.84	6 (7%)	16 (18%)	0.04	4 (5%)	5 (6%)	0.73
Thrombocytopenia	4 (5%)	4 (5%)	1.00	1 (1%)	1 (1%)	1.00	1 (1%)	2 (2%)	0.62
Nausea/Vomiting	31 (35%)	23 (27%)	0.25	3 (3%)	2 (2%)	0.67	1 (1%)	–	0.47
Diarrhea	7 (8%)	4 (5%)	0.53	1 (1%)	–	0.49	1 (1%)	–	0.49
Stipsis	6 (7%)	35 (41%)	<.0001	–	9 (10%)	<.0001	1 (1%)	–	1.00
Mucositis	8 (9%)	4 (5%)	0.37	2 (2%)	4 (5%)	0.68	–	–	–
Alopecia	2 (2%)	1 (1%)	1.00	–	–	–	–	–	–
Transaminases	21 (24%)	12 (14%)	0.06	27 (30%)	20 (23%)	0.14	9 (10%)	11 (13%)	1.00
Fever	4 (5%)	1 (1%)	0.21	4 (5%)	1 (1%)	0.21	–	–	–
Skin toxicity	1 (1%)	4 (5%)	0.20	–	2 (2%)	0.23	1 (1%)	2 (2%)	0.61
Neurological	2 (2%)	33 (38%)	<.0001	–	19 (22%)	<.0001	–	–	–
Infections	–	1 (1%)	0.50	3 (3%)	2 (2%)	1.00	–	–	–
Asthenia	13 (15%)	14 (16%)	0.83	3 (3%)	4 (5%)	0.72	–	–	–
Gastric Pain	5 (6%)	2 (2%)	0.44	1 (1%)	1 (1%)	1.00	–	–	–
Deep venous thrombosis	–	–	–	–	–	–	–	2 (2%)	0.24

C, cyclophosphamide; M, methotrexate; T, thalidomide.

demonstrated via induction of thrombospondin-1, a mediator of antiangiogenic effects [24].

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 metronomic chemotherapy. Besides the interest in the biological efficacy of this regimen, the most relevant aspect related to its use is the demonstration of a 41.5% overall clinical benefit (CR + PR + NC ≥24 weeks), indicating that about half of the patients similar to those who participated in the trial are likely to benefit from this treatment. Moreover, based on activity results shown in a previously published study [5], inclusion of patients not pretreated with systemic chemotherapy for metastatic disease was allowed in the present study. The response rate in this subgroup of patients was 36.1%, with an overall clinical benefit of 55.6%.

No clear evidence of direct cytotoxic effects (e.g. significant myelotoxicity or alopecia) was observed. In fact, in the CM arm only 6% of the patients presented a grade >2 leukopenia or neutropenia, and only 5% had some hair loss. Moreover, the magnitude of the clinical benefit observed was not influenced in the present study by biological features like endocrine responsiveness of the tumor or Her-2/neu overexpression, factors that might influence 'chemoresponsiveness' of breast cancer [25, 26]. These results support the notion that not only the tumor (through a direct cytotoxic effect), but also stromal and vascular compartments, are targets for metronomic chemotherapy.

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 [27], and that VEGF can be considered as a marker of the regulation of angiogenic factors [28]. In the present study, 2 months after treatment, VEGF level had decreased significantly, with equal change in the two arms compared with baseline levels (Table 4).

The administration of thalidomide failed to show additional beneficial effect for the patients. In particular, response rate was not increased with the addition of the drug and in the population of untreated patients a significantly lower response rate was observed for the thalidomide group (9% versus 36%; $P = 0.01$). Despite the lower remission rate, no significant difference in terms of median time to progression (5.7 versus 4 months, arm A and B, respectively; $P = 0.37$) and overall survival (26.2 versus 28.4 months, arm A and B respectively; $P = 0.68$) was observed between the two arms. However, owing to the possible detrimental effect for the concurrent administration of thalidomide with metronomic chemotherapy despite the observed *in vitro* synergic effects [15], we call for caution also in other diseases in which thalidomide is being used (e.g. multiple myeloma) and sometimes associated with cytotoxics [29]. Also, the incidence of several side-effects (neurological grade 1–2, constipation grade 1–2 and leukopenia grade 2) was significantly higher under CM with thalidomide compared with the use of CM alone.

In conclusion, low-dose, oral C and M 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 [30]. The low burden of personal costs to the patient (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), support the use of metronomic CM as an additional therapeutic tool.

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