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Randomized Phase II Trial of Letrozole and Letrozole Plus Low-Dose Metronomic Oral Cyclophosphamide As Primary Systemic Treatment in Elderly Breast Cancer Patients

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ABSTRACT

Purpose
To investigate the activity of letrozole plus/minus oral metronomic cyclophophamide as primary systemic treatment (PST) in elderly breast cancer patients.

Methods
One hundred fourteen consecutive elderly women with T2-4 N0-1 and estrogen receptor–positive breast cancer were randomly assigned to primary letrozole therapy (2.5 mg daily for 6 months) or a combination of letrozole plus oral cyclophosphamide (50 mg/daily for 6 months) in an open-labeled, randomized phase II trial. Tumor response was assessed clinically, and tumor Ki67 index and vascular endothelial growth factor (VEGF) -A levels were measured before and after treatment.

Results
Overall response rate was 71.9% (95% CI, 60.0 to 83.8) in the 57 patients randomly assigned to receive primary letrozole and 87.7% (95% CI, 78.6 to 96.2) in the 57 patients randomly assigned to receive letrozole plus cyclophosphamide. The difference in activity between treatment arms was predominantly confined to patients with ductal histology. There was a significantly greater suppression of Ki67 and VEGF-A expression in the letrozole/cyclophosphamide-treated group than in the letrozole-treated group, leading to lower Ki67 and VEGF expression at post-treatment residual histology ($P = .03$ and $P = .002$, respectively).

Conclusion
Both letrozole and letrozole plus cyclophosphamide treatments appeared active as PST in elderly breast cancer patients. Metronomic scheduling of cyclophosphamide may have an antiangiogenetic effect and the combination of letrozole plus cyclophosphamide warrants testing in a randomized phase III trial.

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INTRODUCTION

Primary systemic antineoplastic therapy (PST) is a means of testing in vivo the sensitivity of breast cancer to novel therapeutic approaches.[1] In hormone receptor–positive postmenopausal women, endocrine therapy can significantly reduce the tumor volume over a 3- to 4-month treatment.[2,3]

Aromatase inhibitors have been shown to be superior to tamoxifen and are being tested extensively as PST in place of tamoxifen.[2,3] Response rates of between 40% and 60% have been reported with aromatase inhibitors in randomized clinical trials.[2,3] Although it is unknown whether clinical response correlates with long-term outcome, very few patients experience progressive disease. These encouraging results may be improved by combining treatments showing synergistic activity as well as drugs able to circumvent endocrine resistance.

Metronomic chemotherapy is the frequent administration of cytotoxic drugs at doses that are low enough to avoid dose-limiting adverse effects, which would otherwise require rest periods.[4] This treatment modality may target tumor cells indirectly via inhibiting angiogenesis and vasculogenesis by continuously exposing the more slowly proliferating tumor endothelial cells to cytotoxic therapy.[5-8] Low-dose metronomic chemotherapy may offer several advantages, including low toxicity and treatment response irrespective of the resistance profile of the tumor cell population.[9-13]

Chemotherapy efficacy is dependent mainly on proliferative activity, whereas endocrine therapies are cytostatic, so that an antagonistic interaction between the two treatment modalities is expected when they are administered concomitantly.[14] The results of a large randomized clinical trial published recently are in line with these assumptions.[15]

Because the target of the metronomic chemotherapy is not the proliferating cancer cells, this treatment modality could potentiate the efficacy of endocrine therapy.

Because of the increased expression of estrogen receptor (ER), the sensitivity of breast cancer to endocrine therapy increases with the age of the patient.[16] Endocrine therapy is therefore the standard treatment for older women both in early and metastatic setting. Because the low toxicity profile of chemotherapy administered on a metronomic schedule makes this modality feasible in the elderly patient population, a randomized phase II trial of letrozole (LET) therapy versus letrozole plus metronomic cyclophosphamide (LET-CYC) as PST in elderly breast cancer patients was conducted at the Breast Unit of Cremona (Italy). The primary aim was to evaluate the activity of the two treatments, with secondary aims to assess the effect of metronomic scheduling on tumor cell proliferative and angiogenetic activity.

METHODS

Patients

Elderly women (age $>$ 70 years) or women between 65 and 70 years of age unfit for chemotherapy with clinical T2-4 N0-1 and ER-positive (ER+) and/or progesterone receptor–positive (PgR+) breast cancer were eligible. They had an Eastern Cooperative Oncology Group performance status of 2 or lower, adequate bone marrow reserve (WBC count, $> 3.5 \times 10^9$/L; platelets, $> 100 \times 10^9$/L; hemoglobin, $>$ 10g/dL), hepatic function (AST/ALT bilirubin and alkaline phosphatase levels $< 1.25\times$ the upper limit of normal value), and renal function (serum creatinine $< 1.25\times$ the upper limit of normal value). Patients with nonmalignant disease that precluded them from receiving study therapy and patients with second primary malignancies were not eligible. The study was approved by the local ethical committee. Written informed consent was obtained from all patients before randomization.

Treatment Schedule

Patients were randomized to receive LET alone, or LET-CYC on a 1:1 ratio. Treatment was started within 1 day of diagnosis. Patients in the LET arm received letrozole (Femara, Novartis, Milan, Italy) 2.5 mg (1 tablet) daily; patients on LET-CYC arm received letrozole and cyclophosphamide (Endoxan, Baxter, Italy) 50 mg (one tablet) daily. These drugs were administered continuously for 6 months until definitive surgery.

Treatment Evaluation and Adverse Effects

On presentation, an incision biopsy was performed on each patient and a small tissue sample (0.5 to 0.8 cm) removed. Each month, the size of the primary tumor was measured with a caliper by the same clinician. Response was assessed according to WHO criteria[17] by the measurement of the changes in the product of the two largest diameters recorded in two successive evaluations. Pathologic complete response (pathCR) was defined as the absence of neoplastic cells in the breast and in the axillary lymph nodes after histologic examination.

Surgery was planned after full clinical reassessment. Quadrantectomy or radical mastectomy was performed when indicated in association with full axillary node dissection.

Toxicity was evaluated according to WHO criteria.[17] No LET reduction was planned. LET was planned to be interrupted in case of severe adverse events.

CYC administration was delayed for the needed time in case of neutrophil count less than 1,500 mm^3 and/or platelet count less than 100,000 mm^3. No dose reduction was adopted. In the event of grade 2 or greater nausea, vomiting, anorexia, increase in transaminases, CYC therapy was interrupted and postponed until symptoms were recovered. Any other adverse effects (grade 3 toxicity) were managed with a postposition of the CYC treatment until full recovery had occurred.

Immunohistochemistry

Immunohistochemical evaluation was performed on paraffin-embedded tumor samples obtained at diagnosis and at definitive surgery. ER, PgR, and Ki67 staining were done at the Pathology Unit of the Azienda Ospedaliera Istituti Ospitalieri of Cremona (Italy), and vascular endothelial growth factor (VEGF) staining was assessed at the John Radcliffe Hospital in Oxford (United Kingdom). The immunohistochemical methodology is fully described elsewhere.[18-20]

Random Assignment and Blinding Procedures

Random assignment was performed using permuted blocks of four assignments in random sequence. The study was open, but the clinician who evaluated all clinical responses (A.Bo.) and the pathologists, either in Cremona or in Oxford, were blinded to treatment assignment.

Statistical Analysis

This randomized trial was treated as two simultaneous phase II studies. The primary end point was the clinical response (complete response [CR] + partial response) rate of each treatment arm among all registered cases (intent-to-treat analysis). LET-CYC was the experimental therapy arm, whereas LET was the standard-therapy arm. On the basis of the activity of primary LET reported in a previous randomized trial,[2] the study was designed to test the null hypothesis that the objective response rate in both arms was less than 60%. According to Simon,[21] a two-stage design was used for both treatment arms to allow early termination of inactive arm(s). With a two-sided alpha of 0.05 and a power of 0.90 to detect a true response probability of 80%, in the first stage, 19 patients were entered onto each arm. If in this stage, more than 12 responses to a treatment have been recorded, the corresponding arm passed to the second stage, with an additional 34 patients recruited. The upper limit of second-stage rejection in each arm was 37 responses observed out of 53 patients enrolled.

To assess the presence of heterogeneity in the effect of the experimental treatment in patient subgroups identified by various prognostic factors (T and N stage, primary histology, tumor grade, PgR status, Ki67, and VEGF expression), a series of logistic regression models were fitted to the data with objective response as dependent variable. The covariates included in each model were treatment and each prognostic factor, one at a time. The presence of a modification of treatment effect in the subgroups identified by each of these factors was assessed by including in the model the appropriate treatment/covariate interaction term(s). The likelihood ratio test was used to evaluate the significance of each interaction term. This procedure is equivalent to a test of the homogeneity of the odds ratios associated with the experimental treatment in the various strata defined by each prognostic factor. Stratum specific odds ratios with their 95% CI are also presented.

Comparison of the distribution of discrete variables in the two treatment arms was performed by the χ^2 or χ^2 for trend when appropriate. For continuous variables, comparisons used the Mann-Whitney U test for nonparametric data. All tests were two sided; $P < .05$ was considered as statistically significant. Statistical analysis was performed on an IBM-compatible personal computer using Statistica software (Statsoft, Tulsa, OK) for Windows (Microsoft, Redmond, WA) software.

RESULTS

From November 2000 to January 2004, 114 patients were enrolled, 57 were randomly assigned to receive LET alone and 57 LET-CYC. Patients' characteristics are outlined in Table 1. As shown in Figure 1, two patients were ineligible but were included in the intent-to-treat

Table 1. Patient Characteristics

	LET		LET-CYC	
	No.	%	No.	%
No. of patients	57		57	
Age, years				
Median	79		75	
Range	64-89		62-94	
PgR status				
Positive	36	64.3	33	57.9
Negative	20	35.7	24	42.1
Not assessable	1		—	
TNM				
T_2	42	73.7	44	77.2
T_{3-4}	15	26.3	13	22.8
N_0	35	61.4	41	71.9
N_1	22	38.6	16	28.1
Primary histology				
Ductal carcinoma	44	77.2	47	82.5
Lobular carcinoma	13	22.8	10	17.5
Grading				
2	22	38.6	22	39.3
3	35	61.4	34	60.7
Not assessable	—		1	
Ki67 expression, %				
≤ 10	24	42.1	19	33.3
11-29	28	49.1	30	52.6
≥ 30	5	8.8	8	14.1
VEGF expression				
1	12	23.1	11	20.8
2	23	44.2	20	37.7
3	17	32.7	22	41.5
Not assessable	5		4	

Abbreviations: LET, letrozole; CYC, cyclophosphamide; VEGF, vascular endothelial growth factor.

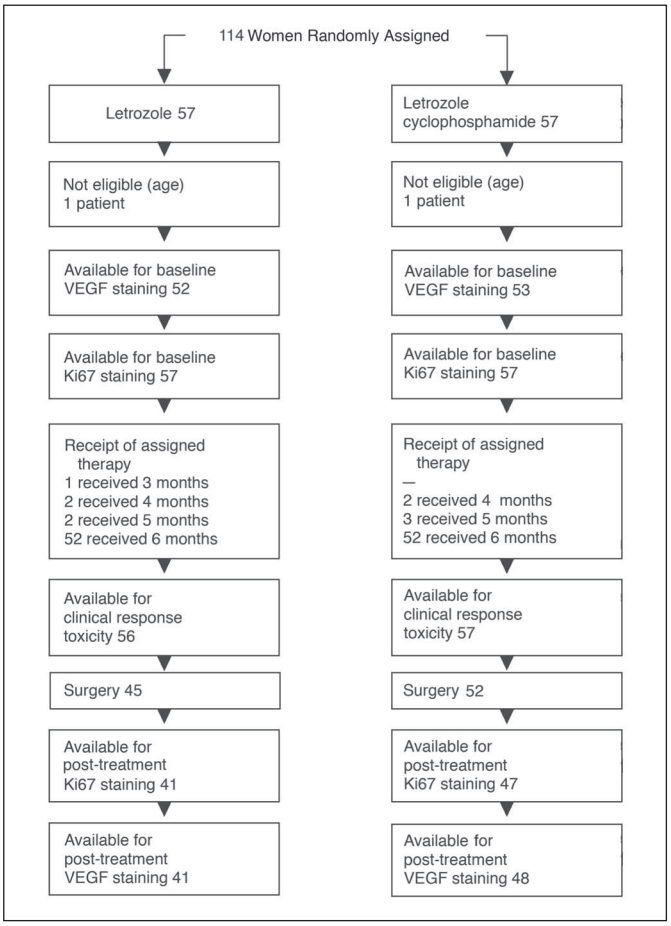

Fig 1. CONSORT diagram. VEGF, vascular endothelial growth factor.

analysis. One hundred four patients (52 in LET and 52 in LET-CYC arm) completed the planned 6 months of therapy, nine were evaluated for response earlier, and one patient was not assessable. The frequency of early treatment discontinuation, resulting from disease progression and patient refusal, did not differ between the treatment arms.

Treatment Toxicity

The most frequent relevant adverse events recorded in both arms were cardiac events and bone-related events. Three patients (two randomly assigned to LET and one to LET-CYC) experienced fatal heart failure after 11, 7, and 6 months from random assignment, respectively. One LET-CYC patient had reversible atrial flutter after 8 months from random assignment. Among bone-related events, three patients (two LET-CYC and one LET) suffered skeletal fractures leading to death in one patient. Three patients (two LET and one LET-CYC) suffered from osteoporotic bone pain. Other adverse events included deep venous thrombosis (one LET-CYC patient), mild (grade 1 and 2) asthenia (two LET patients), and mental impairment (one LET-CYC patient). CYC-related toxicities were grade 4 thrombocytopenia (one patient), grade 2 WBC (two patients), grade 3 cystitis (one patient); grade 2 cystitis (one patient). No patients interrupted nor delayed LET treatment. One patient interrupted CYC administration after 4 months of treatment because of grade 3 cystitis, and one patient delayed CYC for 2 weeks because of grade 4 thrombocytopenia.

Treatment Response

Overall response rate was 50 of 57 (87.7%; 95% CI, 78.6 to 96.2) in the LET-CYC arm and 41 of 57 (71.9%; 95% CI, 60.8 to 83.8), in the LET arm. Complete clinical response, pathCR, and in situ residual carcinoma were similarly distributed between treatment arms (Table 2).

The proportion of responders in the two treatment arms in patient subsets stratified according to baseline clinical and biologic prognostic parameters is shown in Table 3, together with the odds

Table 2. Distribution of Disease Response According to Treatment Arm

	LET		LET-CYC	
	No.	%	No.	%
Not assessable	1	1.7	—	
Progressive disease	3	5.3	3	5.3
Stable disease	12	21.0	4	7.1
Partial response	18	31.6	25	43.8
Complete response	23	40.3	25	43.8
Overall response	41	71.9	50	87.7
95% CI	60.8% to 83.8%		78.6% to 96.2%	
Pathologic response	2	3.5	2	3.5
Residual in situ carcinoma	1	1.8	1	1.8

Abbreviations: LET, letrozole; CYC, cyclophosphamide.

Table 3. Distribution of Response Rates Between the Two Treatment Arms According to Clinical and Biologic Tumor Features

	LET			LET-CYC					
	Total No.	No. Responding	%	Total No.	No. Responding	%	OR	95% CI	*P*
T2	31	42	73.8	38	44	86.4	2.77	0.87 to 8.81	.99*
T3-T4	10	15	66.7	12	13	92.3	2.75	0.43 to 17.49	
N0	24	35	68.6	38	41	92.7	5.81	1.47 to 23.0	.07*
N1	17	22	77.3	12	16	75.0	0.88	0.19 to 3.99	
Ductal carcinoma	32	44	72.7	43	47	91.5	4.03	1.19 to 13.67	.02*
Lobular carcinoma	9	13	69.2	7	10	70.0	1.04	0.17 to 6.23	
Grading 2	17	22	77.3	19	22	86.4	1.86	0.39 to 8.99	.55*
Grading 3	24	35	68.6	30	34	88.2	3.44	0.97 to 12.17	
PgR−	14	20	70.0	22	24	91.7	4.71	0.83 to 26.72	.46*
PgR+	26	36	72.2	28	33	84.8	2.15	0.65 to 7.14	
Ki67 ≤ 10%	20	24	83.3	16	19	84.2	1.07	0.21 to 5.47	.15*
Ki67 > 10%	21		63.6	34	38	89.5	4.86	1.38 to 17.05	
VEGF staining									
1	9	12	75.0	10	11	90.9	3.33	0.29 to 38.08	.92*
2	16	23	69.6	16	20	80.0	1.75	0.43 to 7.17	
3	13	17	76.5	20	22	90.9	3.08	0.49 to 19.28	
Overall	41	57	71.9	50	57	87.7	2.79	1.05 to 7.42	.04†

Abbreviations: LET, letrozole; CYC, cyclophosphamide; OR, odds ratio; PgR, progesterone; VEGF, vascular endothelial growth factor.
*Test for heterogeneity of the ORs of response.
†Comparison of response rate in the two treatment arms.

ratio (OR) of response (LET-CYC *v* LET) in the various strata and in the overall study population. Overall, the combined treatment was associated with a 2.79 (95% CI, 1.05 to 7.42) increased odds of response when compared with LET alone ($P = .04$). No significant heterogeneity in the effect of LET-CYC as compared with LET was observed when analyses were stratified according to T, N, grading, PgR status, Ki67, and VEGF expression. However, a statistically significant interaction between treatment and tumor histology was observed, indicating that the addition of CYC to LET resulted in a more marked activity than LET in patients with ductal carcinoma (OR, 4.03; 95% CI, 1.19 to 13.67; test for interaction $P = .02$).

Ninety-seven patients, 45 randomly assigned to the LET arm and 52 to the LET-CYC arm, underwent surgery. Seventeen patients were not operated on because of patient refusal (15 patients) and death (two patients). At the last follow-up (March 2005), 22 patients progressed and 11 died (median follow-up, 25 months). The proportion of patients alive and disease free after 2 years was 83.5% and 82.0% in the LET and LET-CYC arm, respectively. Thirteen patients were lost to follow-up, seven in the LET and sex in FEM-CYC arm, respectively. They were censored at the date of last follow-up examination.

Changes in Ki67 Expression

Baseline and postchemotherapy Ki67 expression in 88 matched cases is depicted in Figure 2. At baseline, no difference in Ki67 immunostaining between treatment arms was observed. Both LET and LET-CYC treatments resulted in a significant reduction in Ki67 expression before treatment. At postchemotherapy, residual histology Ki67 expression was lower in LET-CYC patients than in LET patients ($P = .03$).

Changes in VEGF Expression

Table 4 displays the distribution of VEGF score between the two arms before and after treatment in 83 matched cases. Although there was no difference in VEGF expression at baseline, VEGF immunostaining was significantly lower in LET-CYC patients than in LET ones ($P < .002$). In particular, 35% positive VEGF tumors at baseline become negative at the end of treatment in LET-CYC arm as opposed to 8% in the LET arm.

Clinical response was observed in 17 of 19 patients attaining a complete VEGF reduction (89.5%), 33 of 40 patients with partial VEGF reduction (82.5%) and in 17 of 24 patients with VEGF no change or increase (70.8%; χ^2 *P* for trend = .12).

DISCUSSION

Endocrine therapy is the optimal PST in elderly breast cancer patients because of the high tumor sensitivity of these patients to endocrine manipulation and the limited compliance of most of them to chemotherapy

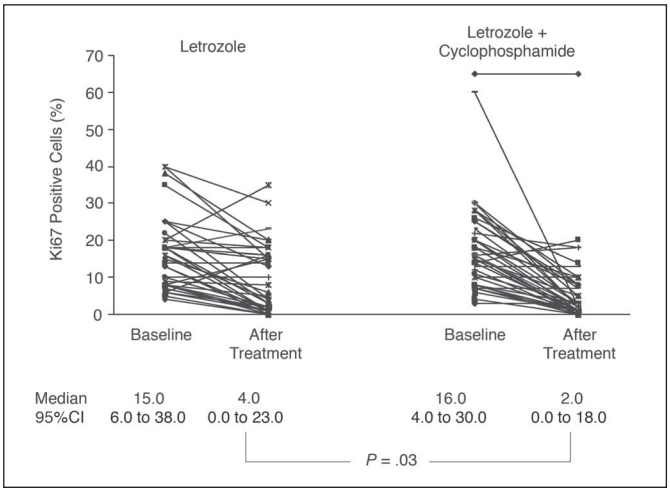

Fig 2. Ki67 expression at baseline and post chemotherapy residual histology. Ki67 expression at post therapy residual histology was significantly lower in patients randomized to receive letrozole plus cyclophosphamide as opposed to those receiving letrozole alone.

Table 4. Changes in VEGF Expression Before and After Treatment According to the Randomized Treatment Arm

	Before				After			
	LET		LET-CYC		LET		LET-CYC	
	No.	%	No.	%	No.	%	No.	%
0	—		—		3	7.9	16	35.5
1	8	21.0	8	17.8	20	52.6	21	46.7
2	18	47.4	17	37.8	12	31.6	8	17.8
3	12	31.6	20	44.4	3	7.9	—	

NOTE. $P < .002$. χ^2 for trend. Post-treatment VEGF 2 and 3 were considered as the same group.
Abbreviations: LET, letrozole; CYC, cyclophosphamide; VEGF, vascular endothelial growth factor.

administered at conventional doses. In this article, we explored the activity of the combination of LET-CYC administration as PST in elderly breast cancer patients as compared to standard LET. The response rate of 72% in patients randomly assigned to the LET arm was higher than the 60% obtained in a previous randomized trial with primary LET therapy.[2] The different patient population and the longer exposure of our patients to LET (6 months *v* 4 months) can account for the observed difference.

The response rate obtained in the LET-CYC arm (88%) was high. The study design was not aimed at testing the difference in response rates in the two treatment arms, and both passed the test of activity. However, the comparison with the randomized control arm indicates that the high activity of the experimental arm was not caused by a biased sample,[22] and suggests that the addition of CYC is associated with an increase in the activity of LET in this patient population (OR, 2.79). Although these results are encouraging, they failed to be confirmed by pathCR, a known predictor of long-term outcome. pathCR was observed in two patients (3.5%), one in each arm. A very low pathCR with primary LET therapy (1.7%) was obtained in the randomized trial comparing LET versus tamoxifen,[2] suggesting that this condition is not a sensitive end point for primary endocrine therapy. The addition of metronomic CYC failed to increase the pathCR rate. Others have also found that patients with ER+ tumors have a low propensity to obtain pathCR after chemotherapy.[23,24]

The exploratory subgroup analyses according to baseline prognostic parameters, suggest that the increased activity associated with the addition of CYC to LET seems to be more marked in (or confined to) patients with ductal histology.[25] Even though caution should be adopted in interpreting data coming from subgroup analyses, one might speculate that the addition of metronomic chemotherapy in this subset of patients could improve treatment results.

The efficacy of chemotherapy administered at conventional doses is mainly dependent on actively proliferating cells that might be countered by concomitant endocrine therapy that reduces tumor proliferative activity.[15] However, because the target of metronomic chemotherapy is not the proliferative compartment,[4] the coadministration of metronomic chemotherapy with endocrine therapy should not interfere but enhance the efficacy of the combination.

In a randomized neoadjuvant trial comparing anastrozole versus tamoxifen versus the combination of both drugs (Immediate Preoperative Anastrozole Tamoxifen or Combined with Tamoxifen [IMPACT]),[26] it was shown that reduction in Ki67 after anastrozole was greater than that obtained with either tamoxifen or the combination. Mean changes in Ki67 in the IMPACT trial were reflected in differences in disease-free survival in the Arimidex Tamoxifen and Combination (ATAC) adjuvant trial,[27] suggesting that reduction in Ki67 after endocrine therapy is predictive of long-term outcome. In the present study the addition of metronomic CYC to LET led to a greater reduction in proliferative activity as assessed by changes in percent of Ki67-positive tumor cells before and after treatment, further supporting the potential synergy between the two treatments. The target of metronomic therapy is the endothelial cell, but the additional effect on Ki67 in tumor cells could have been indirect, because of effects on vessels. The more proliferative tumors have a higher oxygen demand and may be more sensitive to an antivascular component. It is also possible that even this low-dose of CYC has a direct effect on tumor cell proliferation.

The antiangiogenetic effect of metronomic chemotherapy has been evaluated rarely in the clinic. To our knowledge, this is the first randomized PST trial evaluating the effects on VEGF directly in the tumor. In our series, LET showed a modest reduction in VEGF expression, probably mediated by estrogen deprivation.[28] The addition of CYC resulted in a greater reduction of VEGF immunostaining than LET alone, leading to a lower VEGF expression at post-treatment residual disease. Probably because of the limited sample size of the study, the relationship between disease response and changes in VEGF immunostaining failed to attain statistical significance. It should be noted, however, that the efficacy of an antiangiogenetic drug may not necessarily be linked to the disease response obtained.

One mechanism by which metronomic therapy has been shown to work is reduction of endothelial proliferation, and another through inhibition of mobilization of bone marrow endothelial progenitor cells, both of which are partly regulated by VEGF.[29,30] Our results suggest that inhibition of production of VEGF in the tumor may be one mechanism by which such therapy works on both targets. Whether this effect would occur with CYC alone cannot be assessed in this trial, and it will be of interest to evaluate effects on other angiogenetic factors.

Nevertheless, although long-term skeletal and cardiac toxicities of LET therapy are recognized,[31] in this trial few patients experienced heart failure and bone fractures. The age of patients experiencing these adverse events was 79 years, close to average life expectancy, and treatment exposure was relatively short, so that it is difficult to state whether the events should be attributed to LET administration. The uniform distribution of events between the treatment arms, however, suggest that they were not influenced by concomitant CYC administration. The adverse effects attributable to CYC were modest and rarely dose limiting.

In conclusion, concomitant administration of metronomic CYC and LET appears to be a feasible and active PST in elderly breast cancer patients. These results warrant testing the combination LET-CYC against LET in a phase III study.

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The authors indicated no potential conflicts of interest.

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