Cancer Chemother Pharmacol (1996) 38: 487–494

ORIGINAL ARTICLE

**Marco Venturini · Lucia Del Mastro · Franco Testore
Marco Danova · Ornella Garrone · Claudio
Lanfranco · Fabio Latini · Mario R. Sertoli
Rita Lionetto · Paola Queirolo · Andrea Ardizzoni
Riccardo Rosso**

Erythropoietin and granulocyte-macrophage colony-stimulating factor allow acceleration and dose escalation of cyclophosphamide/epidoxorubicin/5-fluorouracil chemotherapy: a dose-finding study in patients with advanced breast cancer

Received: 8 October 1995/Accepted: 1 March 1996

Abstract To verify whether the association of granulocyte-macrophage colony-stimulating factor (GM-CSF) and erythropoietin (EPO) would allow both the acceleration and the dose escalation of the cyclophosphamide/epidoxorubicin/5-fluorouracil (CEF) regimen as first-line therapy in advanced breast cancer patients, we conducted a dose-finding study. Cohorts of three consecutive patients received cyclophosphamide (Ctx, dose range 800 –1400 mg/m^2), epidoxorubicin (Epidx, dose range 70–100 mg/m^2), and 5-fluorouracil (5-Fu, 600 mg/m^2, fixed dose) given as an intravenous bolus on day 1 every 14 days; GM-CSF at 5 μg/kg given as a subcutaneous injection from day 4 to day 11; and EPO at 150 IU/kg given as a subcutaneous injection three times a week. In no single patient was any dose escalation allowed. A total of 14 patients entered the study. At the 4th dose level (Ctx 1400 mg/m^2, Epidx 100 mg/m^2, 5-Fu 600 mg/m^2), two patients had dose-limiting mucositis and one patient developed dose-limiting neutropenia. Therefore, the 3rd cohort received the maximum tolerated dose, i.e. Ctx at 1200 mg/m^2, Epidx at 90 mg/m^2, and 5-Fu at 600 mg/m^2, given every 18.5 (± 2.5) days. Toxicity was moderate and manageable in an outpatient setting. Only 1 admission at the 4th dose level was required. Throughout the 4 dose levels there was no toxicity-related death; grade IV leukopenia ranged from 24% to 75% of cycles and grade IV thrombocytopenia ranged from 6% to 8%. No grade IV anemia was recorded. Increasing the doses of Ctx and Epidx while maintaining a fixed dose of 5-Fu with the support of both EPO and GM-CSF allows safe acceleration and dose escalation of CEF chemotherapy. Further controlled studies will evaluate the activity and efficacy of this strategy.

Key words Breast cancer · CEF · Dose intensity · Erythropoietin · GM-CSF

This work was presented in part at the 1995 meeting of the American Society for Clinical Oncology

M. Venturini (✉) · L. Del Mastro · O. Garrone · P. Queirolo
A. Ardizzoni · R. Rosso
Oncologia Medica 1, Istituto Nazionale per la Ricerca sul Cancro, Viale Benedetto XV 10, I-16132 Genova, Italy

F. Testore · C. Lanfranco
Unita Operativa Oncologia, Ospedale Civile, Via Botallo 4, I-14100 Asti, Italy

M. Danova
Medicina Interna e Oncologia Medica, Università e IRCCS San Matteo, Piazzale Golgi, I-27100 Pavia, Italy

F. Latini
Shering-Plough Italia, Direzione Medica, Via Ripamonti 89, I-20141 Milano, Italy

M.R. Sertoli
Istituto di Oncologia, Università, Viale Benedetto XV 10, I-16132 Genova, Italy

R. Lionetto
Epidemiologia Clinica e Sperimentazioni Cliniche Controllate, Istituto Nazionale per la Ricerca Sul Cancro, Viale Benedetto XV 10, I-16132 Genova, Italy

Introduction

The importance of dose has received a resurgence in interest in the last 10 years due to both the extensively debated concept of dose intensity introduced by Hryniuk and Bush in 1984 [1] and the more recent clinical availability of hematopoietic colony-stimulating factors. To define the importance of dose, several studies have been conducted in breast cancer patients. Reducing the dose below standard levels has been proven to be detrimental in both metastatic [2] and

early-stage [3] breast cancer. Augmenting the dose over standard levels may be beneficial, at least in terms of the response rate, but there is no clear evidence of any improvement in survival [4]. This could be related to the small increase in the dose rate or in the dose actually given in 11 randomized studies that have tested the role of dose in advanced breast cancer [4].

To date the most important factor actually limiting the administration of higher than standard doses has been hematological toxicity. Granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) have been proven to reduce the depth of leukocytes' nadir and to shorten the duration of leukopenia after standard chemotherapy [5, 6].

We have demonstrated that with the use of GM-CSF [7] or G-CSF [8] the dose rate of the combination of cyclophosphamide, epidoxorubicin and 5-fluorouracil (CEF regimen) can be augmented; the interval between cycles can be reduced from 21 days to 14–17 days. This accelerated regimen led to increased myelosuppression that could only partially be counteracted by GM-CSF or G-CSF; cumulative anemia and thrombocytopenia became the main hematological toxicities.

Erythropoietin (EPO) has been shown to be capable of raising hemoglobin concentrations both in patients with renal failure and in those with acquired immunodeficiency syndrome (AIDS), and it is also effective in the treatment of chemotherapy-induced anemia [9]. The rationale of the addition of EPO to GM-CSF was the possibility of both reducing the incidence of anemia with EPO and shortening the neutropenic period with GM-CSF. In patients with aplastic anemia a beneficial effect has been suggested by use of the association of GM-CSF and EPO on both anemia and neutropenia, with no increase in toxicity being observed [10]. A beneficial clinical interaction between EPO and GM-CSF has also been reported after autologous bone marrow transplantation [11]. A possible capability of the combination itself to reduce the incidence of thrombocytopenia was also foreseen. Data on subhuman primates showed a substantial increase in the platelet count when GM-CSF and EPO were given sequentially as compared with a minimal increase when GM-CSF was given alone [12].

On this basis we started a dose-finding study to verify the possibility of increasing the dose of an accelerated CEF regimen in an outpatient setting by the addition of a combination of EPO and GM-CSF.

Patients and methods

Patient selection

Eligible patients were women with clinical or histological evidence of locally advanced (stage III) or metastatic breast cancer [13] who had to have either measurable or evaluable disease as well as a WHO performance status of ≤ 1 and had to be nonpregnant and nonlactating. Other eligibility criteria were the absence of previous or concomitant malignancy and no other serious medical or psychiatric illness that would prevent informed consent or preclude intensive treatment. Prior adjuvant chemotherapy was allowed if completed at least 12 months before study entry. Any prior hormonetherapy, either as adjuvant treatment or for metastatic disease, was acceptable. A white blood cell count (WBC) of $\geq 3000 \times 10^6$/l and/or an absolute neutrophil count (ANC) of $> 2000 \times 10^6$/l a platelet (PLT) count of $\geq 100 \times 10^9$/l, a hemoglobin value (Hb) of ≥ 10g/dl, a creatinine level of $\leq 1.5 \times$ normal, a blood urea nitrogen value of $\leq 1.5 \times$ normal, and normal albumin and bilirubin levels were also required. Criteria for exclusion were a history of congestive heart failure, myocardial infarction, angina, or serious cardiac arrhythmia; a history of chronic liver disease; clinical evidence of brain metastases; prior chemotherapy or extensive radiotherapy (estimated to involve more than 30% of the bone marrow) for metastatic disease; any prior cytokine therapy; and concomitant radiotherapy to any site. Each patient gave her written informed consent.

Treatment plan

Patients received accelerated and dose-escalated CEF chemotherapy consisting of cyclophosphamide (Ctx), epidoxorubicin (Epidx), and 5-fluorouracil (5-Fu) plus GM-CSF and EPO according to the following design. Ctx at 800 mg/m^2 (starting dose), Epidx at 70 mg/m^2 (starting dose), and 5-Fu at 600 mg/m^2 (fixed dose) were given by intravenous bolus on day 1. GM-CSF at 5 μg/kg was self-administered by subcutaneous injection from day 4 to day 11. As in the previous study [7], the choice of day 4 as the first day of administration of GM-CSF was based on the long duration for plasma clearance of Epidx. Starting on the 1st day of chemotherapy, EPO at 150 IU/kg was self-given by subcutaneous injection three times a week until 2 weeks after completion of the last cycle of chemotherapy.

Chemotherapy was repeated every 2 weeks if the WBC was $\geq 3000 \times 10^6$/l and/or the ANC was $\geq 2000 \times 10^6$/l and the PLT count was $\geq 100 \times 10^9$/l. Otherwise, WBC and/or ANC and PLT values were assessed daily until recovery, then chemotherapy was given. If no dose-limiting toxicity (DLT) occurred (as defined below), the doses of Epidx and Ctx were increased by consecutive cohorts of three patients as follows:

Dose (mg/m^2)	Cohort			
	1st	2nd	3rd	4th
Ctx	800	1000	1200	1400
Epidx	70	80	90	100
5-Fu	600	600	600	600

Patients treated at a given dose were observed throughout three courses of chemotherapy before entry at the next dose level began. If one patient treated at a given dose experienced DLT, a maximum of three additional patients were entered at the dose. If no more than two of six patients experienced DLT, escalation was resumed. If three patients experienced DLT at a given dose, no further escalation was allowed. The previous dose should have been declared the maximum tolerated dose (MTD).

No intrapatient chemotherapy dose escalation or dose reduction was allowed. If grade III GM-CSF-related toxicity occurred, the cytokine was first withdrawn until resolution of the adverse reaction and then resumed at a dose of 3 μg/kg. When, despite the dose reduction, toxicity persisted, GM-CSF was definitively stopped.

GM-CSF was temporarily suspended if the WBC was $\geq 50\,000 \times 10^6$ /l. EPO was stopped if the Hb value was ≥ 15 g/dl in two consecutive blood tests conducted 7 days apart. EPO was resumed when the Hb value was 13 g/dl.

Paracetamol was given if required to control bone pain or fever related to GM-CSF treatment. Red blood cell transfusions were given if the Hb value fell ≤ 8 g/dl. Prophylactic administration of ciprofloxacin at 500 mg b.i.d. was recommended in patients with a WBC of $< 1000 \times 10^6$/l and/or an ANC of $< 500 \times 10^6$/l. Platelet transfusions were given if the PLT count fell to $\leq 20 \times 10^9$/l.

A complete medical history and physical examination with weight and height measurement, determination of the performance status, complete biochemical tests, a complete blood count with WBC differential, an ECG, a chest X-ray, an abdominal ultrasound and/or computerized tomography (CT) scan, a bone scan and/or bone X-rays, and other examinations, if clinically indicated, were required prior to study entry.

Prior to each cycle, complete biochemical tests, nonhematological toxicity evaluation, and tumor measurement by physical examination were performed. A complete blood count was done twice a week. Every other cycle, tumor measurement was carried out using the same initial imaging technique. Although response was not the primary objective in this study, standard criteria were used for determination of the response to therapy [14]. The study was approved by the Protocol Review Committee and Ethics Committee of the Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy.

Toxicity

Hematological toxicity was recorded at nadir. Toxic effects were graded using WHO criteria [14]. If, for a given symptom, no WHO grade was described, the toxicity grade was recorded as follows: I mild, II moderate, III severe, and IV life-threatening. DLT was defined as grade III–IV nonhematological toxicity (excluding nausea, vomiting, fever, anorexia, alopecia, and grade III stomatitis and esophagitis-dysphagia lasting for 3 days or less), grade III stomatitis and esophagitis-dysphagia lasting for longer than 3 days, grade IV leukopenia and/or neutropenia or thrombocytopenia lasting 7 or more days, failure of WBC or PLT counts to recover by day 21, grade IV neutropenia associated with clinically documented infection, grade IV thrombocytopenia associated with bleeding, and any grade IV toxicity related to GM-CSF treatment.

Dose-intensity calculation and statistics

Dose intensity, expressed in milligrams per square meter of body surface area per week, was calculated for each drug by dividing the total amount of drug received by the duration of chemotherapy. This time was calculated by adding the mean interval for each patient, as the interval after the last cycle of chemotherapy, to the actual time taken. The relative dose intensity (RDI) and average relative dose intensity (ARDI) were calculated according to Hryniuk and Bush [1]. As the reference standard regimen we selected CEF chemotherapy at 600 /60/600 mg/m^2, which was repeated every 21 days. Patients receiving at least two cycles of CEF chemotherapy were evaluated for dose-intensity calculation. Data are reported as mean values with standard deviations being given in parentheses.

Results

A total of 14 women entered the study. Their main characteristics are shown in Table 1. All patients had a performance status of 0. No patient had received previous hormonal therapy for metastatic disease.

Table 1 Main characteristics of the patients

	Number of patients
Total number of patients	14(100%)
Median age (years)	53.5
Range	34–67
Stage:	
IIIb	3(21%)
IV	11(79%)
Dominant site of metastases[a]:	
Visceral ± other sites	6(55%)
Soft tissue ± bone	3(27%)
Bone alone	2(18%)
Prior adjuvant chemotherapy[a]:	
Yes	4(36%)
No	7(64%)

[a] In patients with stage IV breast cancer

Four patients were premenopausal and ten were postmenopausal. Three patients with locally advanced disease received accelerated escalated CEF as neoadjuvant chemotherapy; thereafter they underwent radical surgery, treatment with standard-dose CEF as adjuvant chemotherapy, and, finally, local radiotherapy.

Overall, 69 cycles of chemotherapy were given. In all, 39 (56.5%) cycles had to be delayed: 26 due to leukopenia, 4 due to leukopenia plus thrombocytopenia, 4 for organizational reasons, 2 because of fever, 2 due to the patients' decision, and 1 for cystitis. Excluding level 4, in which DLT was observed, a median of 5 (range 4–8) cycles were given. Table 2 shows the number of cycles received, the mean interval, and the average relative dose intensity recorded for each patient.

When all 14 patients were considered, regardless of the assigned cohort, the mean interval between cycles was 16.5 (±2.1) days. We observed a progressive increase throughout the cycles of chemotherapy; the mean interval at the 1st and the 5th cycle was 15.6 (±2.3) and 18.5 (±2.0) days, respectively. Excluding the 4th cohort, in which three of four patients received only 2 cycles of chemotherapy, the mean interval between cycles was 17.4 (±1.7) days. The increase in dose led to a progressive increase in the mean interval between cycles: 16.8 (±2.1), 17.1 (±2.7) and 18.1 (±2.5) days in the 1st, 2nd, and 3rd cohort, respectively. The objective response rate was 78.6% (95% confidence interval 49.2–95.3%). Table 2 shows the best response obtained for each patient.

Toxicity

There was no toxicity-related death. Only one patient required hospitalization. Three patients treated at the

Table 2 Patients' outcome (*CR* Complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *NE* not evaluable)

Patient number	Dose level	Number of cycles received	Mean interval between cycles (days)	Ratio of received/planned dose intensity	Objective response
1	1st	5	18.8	0.73	PR
2	1st	8	16.4	0.85	PR
3	1st	5	15.3	0.92	PR
4	2nd	6	15.4	0.91	PR
5	2nd	8	18.9	0.74	PR
6	2nd	4	17.0	0.77	PD
7	3rd	4	16.3	0.96	CR
8	3rd	4	18.7	0.75	PR
9	3rd	8	20.3	0.69	CR
10	3rd	5	17.0	0.82	PR
11	4th	2	14.0	0.94	NE
12	4th	6	14.4	0.90	CR
13	4th	2	14.0	0.94	SD
14	4th	2	14.0	1.00	PR

Table 3 Nonhematological toxicity encountered

Toxicity: grade	Number of cycles (%)[a]			
	1st cohort (total 18 cycles)	2nd cohort (total 18 cycles)	3rd cohort (total 21 cycles)	4th cohort (total 12 (cycles)
Nausea/vomiting:				
0	15 (83)	5 (28)	8 (38)	5 (42)
I	1 (6)	11 (61)	7 (33)	5 (42)
II	1 (6)	2 (11)	4 (19)	–
III	1 (6)	–	2 (10)	2 (17)
IV	–	–	–	–
Stomatitis:				
0	14 (78)	12 (67)	16 (76)	6 (50)
I	2 (11)	1 (6)	3 (14)	2 (17)
II	2 (11)	5 (28)	2 (10)	2 (17)
III	–	–	–	1 (8)
IV	–	–	–	1 (8)
Fatigue:				
0	8 (45)	11 (61)	15 (72)	9 (75)
I	–	3 (17)	–	–
II	9 (50)	2 (11)	2 (10)	1 (8)
III	1 (6)	2 (11)	4 (19)	2 (17)
Anorexia:				
0	14 (78)	14 (78)	16 (76)	9 (75)
I	–	2 (11)	–	–
II	3 (17)	–	3 (14)	1 (8)
III	1 (6)	2 (11)	2 (10)	1 (8)
IV	–	–	–	1 (8)
Fever:				
0	16 (89)	14 (78)	16 (76)	9 (75)
I	1 (6)	1 (6)	1 (5)	1 (8)
II	1 (6)	3 (17)	4 (19)	–
III	–	–	–	1 (8)
IV	–	–	–	1 (8)
Bone pain:				
0	18 (100)	14 (78)	14 (67)	10 (83)
I	–	3 (17)	7 (33)	–
II	–	–	–	2 (17)
III	–	1 (6)	–	–

[a] Because of rounding, percentages may not total 100

4th dose level experienced DLT. Patient 11 had grade IV mucositis and was admitted, patient 13 had grade IV fever and grade IV neutropenia lasting for 7 days and was treated with outpatient parenteral antibiotic therapy, and patient 14 had grade III mucositis lasting for 4 days. Therefore, the dose level for the 3rd cohort was declared the MTD.

The main nonhematological toxicities encountered are listed in Table 3. All patients had grade III alopecia. Patient 9 suffered from mild cystitis at the 1st cycle and patient 3 had moderate cystitis at the 4th cycle. The most relevant nonhematological toxicities, regardless of the grade, were fatigue in 8 patients and mucositis in 9 patients. Whereas mucositis was the DLT in 2 patients in the 4th cohort and was probably dose-related, no clear dose-effect relationship was observed for fatigue.

GM-CSF-related toxicity was mild or moderate in most patients. Of 13 patients, 11 required no dose reduction or suspension. Patient 6 had grade III bone pain at the 4th cycle but stopped therapy due to progressive disease. Patient 10 self-suspended administration of GM-CSF for 2 days during cycles 1 and 2; at the 5th cycle she stopped GM-CSF therapy for 1 day and then resumed therapy at half the dose. Cutaneous reactions, either rash or injection-site reaction, were recorded in 4 patients. One patient developed a "first-dose reaction" as described by Lieschke et al. [15] but continued GM-CSF in subsequent cycles at the same dose without any problem. EPO-related side effects were not observed.

The main hematological toxicities recorded at nadir in 67 of 69 cycles are listed in Table 4. No patient required platelet transfusion. Only patient 7 received 4 units of red blood cell transfusions. Neutropenic fever managed in an outpatient setting was recorded in 3 cases (patients 4, 5, and 6) and in 6 (9%) cycles. Patients 5, 8, 9, and 13 received oral antibiotic prophylaxis for 17 (25%) cycles due to grade IV leukopenia or neutropenia. The course of WBC, PLT, and Hb nadir counts throughout 5 cycles is shown in Fig. 1. Excluding the 4th level, in which the DLT was observed, the mean nadirs of WBC (Fig. 1a) and PLT (Fig. 1b) were not particularly affected by either the dose level or the cycle of chemotherapy. The mean Hb nadir was also unrelated to the dose level (Fig. 1c), although it worsened progressively with increasing cycles of chemotherapy. For the 1st, 2nd, and 3rd cohorts the mean Hb nadir was 10.9 ($\pm$0.4), 11.8 ($\pm$2.0), and 12.6 ($\pm$0.7) g/dl, respectively, after the 1st cycle and decreased to 9.9 ($\pm$0.6), 9.9 ($\pm$0.7), and 10.6 ($\pm$0.1) g/dl, respectively, after the 5th cycle (Fig. 1c).

Dose-intensity results

Patients treated at the 1st dose level received 83% of the planned ARDI and those treated at the 2nd and 3rd dose levels received 81%. This means a respective 46%, 61%, and 75% actual dose-intensity increase as compared with a standard dose of CEF given every 21 days. This was mainly obtained by increasing the doses of Ctx and Epidx (Table 5). The actual dose-intensity increase of Ctx progressed from 65% at the 1st dose level to 134% at the 3rd dose level, and that of Epidx progressed from 45% to 75%. In contrast, due to the progressive lengthiness of intervals between cycles the 5-Fu dose-intensity gradually decreased (Table 5).

Table 4 Hematological toxicity encountered at nadir

Toxicity: grade	Number of cycles (%)[a]			
	1st cohort (total 17 cycles)	2nd cohort (total 17 cycles)	3rd cohort (total 21 cycles)	4th cohort (total 12 (cycles)
Leukopenia:				
0	–	–	2 (10)	–
I	2 (12)	2 (12)	1 (5)	–
II	5 (29)	1 (6)	2 (10)	1 (8)
III	6 (35)	10 (59)	8 (38)	2 (17)
IV	4 (24)	4 (24)	8 (38)	9 (75)
Anemia:				
0	4 (24)	6 (35)	13 (62)	5 (42)
I	8 (47)	7 (41)	7 (33)	3 (25)
II	4 (24)	3 (18)	1 (5)	3 (25)
III	1 (6)	1 (6)	–	1 (8)
Thrombocytopenia:				
0	13 (77)	11 (65)	14 (67)	8 (67)
I	2 (12)	5 (29)	2 (10)	–
II	1 (6)	1 (6)	1 (5)	2 (17)
III	–	–	2 (10)	1 (8)
IV	1 (6)	–	2 (10)	1 (8)

[a] Because of rounding, percentages may not total 100

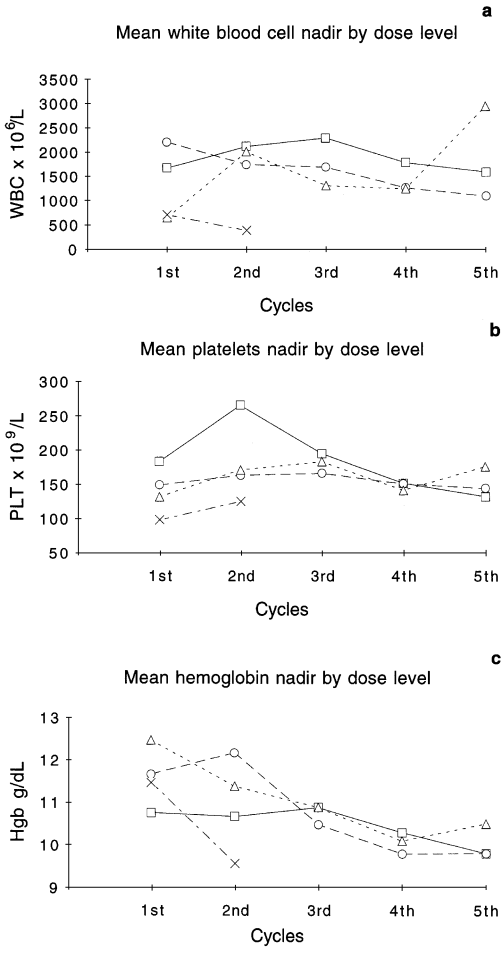

Fig. 1a–c Mean WBC (**a**), PLT (**b**), and Hgb (**c**) nadir counts recorded during 5 cycles of CEF chemotherapy (*squares* Ist level, *circles* 2nd level, *triangles* 3rd level, *x* 4th level)

Table 5 Dose-intensity results recorded for the first 3 cohorts

	Ist cohort		2nd cohort		3rd cohort	
	DI[a]	RDI[b]	DI[a]	RDI[b]	DI[a]	RDI[b]
Ctx	330	1.65	403	2.02	467	2.34
Epidx	29	1.45	32	1.60	35	1.75
Fu	253	1.27	242	1.21	234	1.17
Mean	204	1.46	226	1.61	245	1.75

[a] Actual delivered dose intensity (mg/m^2/week)
[b] Relative dose intensity (see Patients and methods for calculation of RDI)

Discussion

The clinical availability of hematopoietic colony-stimulating factors has enabled the initiation of clinical trials to verify the possibility of augmenting the dose rate and the dose of some anticancer drugs. Among the possible ways of increasing the dose rate, our group has tried to shorten the interval between chemotherapy cycles, i.e., to accelerate the chemotherapy. We have demonstrated that some regimens commonly used in small-cell lung cancer [16] and breast cancer [7, 8] patients can be safely accelerated with the use of GM-CSF or G-CSF. Particularly in breast cancer patients, using GM-CSF we demonstrated that standard-dose CEF chemotherapy can be given at a mean interval of 17.3 days. The main toxicities encountered were fatigue, cumulative anemia, and thrombocytopenia. Interestingly, a comparison of accelerated versus standard CEF therapy revealed an increase from 42% to 69% in the objective response rate, which had borderline statistical significance [7]. Therefore, we concluded that increasing the dose rate in standard-dose CEF treatment was a new and effective approach in the treatment of advanced breast cancer. The next rational step was to verify whether the dose increase for the accelerated CEF regimen was also feasible. The study reported herein was aimed at clarifying this issue.

We decided to maintain the fixed dose of 5-Fu but to increase progressively the doses of Ctx and Epidx on the basis of the activity and toxicity of each drug. Indeed, Ctx and Epidx are among the most active single drugs used in the treatment of metastatic breast cancer, producing an objective response rate of 32% and 34%, respectively [17]. In contrast, although 5-Fu is one of the most commonly used drugs in metastatic breast cancer, it is rarely used in monochemotherapy because its activity is lower than that of Ctx or Epidx, i.e., a 27% objective response rate [17]. Moreover, the increase in the clinical activity of 5-Fu obtained by dose escalation or by biochemical modulation led to the development of significant toxicity [18].

The main objective of our study was to augment the dose intensity of the accelerated CEF regimen by increasing the doses. As compared with a standard CEF regimen, a substantial increase in the delivered dose intensity was actually obtained, amounting to 61% in the 2nd cohort and 75% in the 3rd. Few studies using GM-CSF to increase the dose intensity of breast cancer chemotherapy have been fully reported. NSABP reported a pilot study in which advanced breast cancer patients were treated with a fixed dose of doxorubicin (Dx) and escalated Ctx doses of up to 2.4 g/m^2 every 21 days. Only 2 cycles of chemotherapy were foreseen. Therefore, no meaningful conclusion can be drawn about its actual usefulness to patients with advanced breast cancer who definitively require more than 2 cycles. Moreover, on treatment with these doses, 80% of the patients had to be admitted [19].

Two other studies substantially failed to increase the dose intensity of standard regimens with the support of GM-CSF. Hoekman et al. [20] treated 18 advanced breast cancer patients with escalating doses of Dx and Ctx given every 3 weeks. At the 2nd level (Dx 90 mg/m^2, Ctx 1000 mg/m^2), dose-limiting stomatitis, cumulative thrombocytopenia, and neutropenia occurred. This toxicity, worse than that observed at our 3rd

level (Ctx 1200 mg/m^2, Epidx 90 mg/m^2, 5-Fu 600 mg/m^2), may be explained by the use of the high dose of Dx. Indeed, 90 mg/m^2 of Dx can be considered equitoxic to 135 mg/m^2 of Epidx [21].

Recently, a phase I/II study indicated that FLAC (5-Fu, leucovorin, Dx, and Ctx) plus GM-CSF was a very active but also very toxic regimen. In all, 98% of the patients had grade IV neutropenia; 79%, grade IV thrombocytopenia; 94%, grade III–IV anemia; and 32%, grade III–IV mucositis. This study differs from ours essentially in the choice of drugs to be intensified. Ctx and Dx doses were not escalated, whereas a clear intensification of the 5-Fu dose was obtained. In addition, the action of 5-Fu was enhanced by the use of leucovorin [18].

In our study, hematological toxicity was moderate and no cumulative toxicity to WBC or platelets was observed. Indeed, the mean WBC and PLT nadirs were not significantly modified throughout the cycles of chemotherapy (Fig. 1a, b). The lack of cumulative toxicity to platelets differs from the finding of our previous study [7]. In the latter study, patients treated with standard-dose accelerated CEF with the support of GM-CSF alone experienced progressive cumulative thrombocytopenia, which was life-threatening (grade IV) in 14% of the patients. On the other hand, in the present as well as the previous study, anemia progressively worsened with increasing cycles of chemotherapy. However, as expected, with the use of EPO the incidence of grade III–IV anemia was lower (4.5% of cycles in the present study versus 12.6% in the previous one).

Increasing the doses led to an increase in non-hematological toxicity. Stomatitis was the most relevant toxicity. All patients in the 4th cohort suffered from this side effect, and in two patients it was the DLT. The development of severe mucositis is related to the use of anthracyclines at a higher than standard dose intensity [22]. Fatigue was also a frequent side effect, being observed in 8 (57%) of 14 patients. This side effect could conceivably be related to the use of GM-CSF [23]. However, in a study such as ours it is difficult to separate the effect of accelerated escalated chemotherapy from the effect of GM-CSF.

In conclusion, the strategy of increasing the doses of CTX and Epidx and maintaining the fixed dose of 5-Fu led to a feasible increase in the dose intensity of CEF along with manageable toxicity; CTX at 1200 mg/m^2, Epidx at 90 mg/m^2, and 5-Fu at 600 mg/m^2 can be given as an outpatient regimen every 18–19 days. Taking into account that hematopoietic growth factors are expensive molecules, the question as to whether their support is cost-effective is a matter for future controlled clinical trials, which should mainly evaluate the eventual increase in activity and efficacy.

Acknowledgements This work was partly supported by a grant from the Progetto Finalizzato Ministero della Sanitá 1993, ICS 34.2/RF93.58, and by CNR grant 94.01242-PF39 (ACRO).

References

1. Hryniuk W, Bush H (1984) The importance of dose intensity in chemotherapy of metastatic breast cancer. J Clin Oncol 2:1281–1288
2. Tannock IF, Boyd NF, DeBoer G, Erlichman C, Fine S, Laroque G, Mayers C, Perrault D, Sutherland H (1988) A randomized trial of two dose levels of cyclophosphamide, methotrexate and fluorouracil chemotherapy for patients with metastatic breast cancer. J Clin Oncol 6:1377–1387
3. Wood WC, Budman DR, Korzun AH, Cooper MR, Younger J, Hart RD, Moore A, Ellerton JA, Norton L, Ferree CR, Colangelo Ballow A, Frei E III, Henderson C (1994) Dose and dose intensity of adjuvant chemotherapy for stage II, node-positive breast carcinoma. N Engl J Med 330:253–259
4. Venturini M, Del Mastro L, Ardizzoni A, Sertoli MR, Mariani GL, Pennucci MC, Garrone O, Silvestro S, Addamo GF, Queirolo P, Rosso R (1993) High dose intensity chemotherapy without bone marrow support: role of granulocyte-macrophage colony-stimulating factor. Ann NY Acad Sci 698:389–397
5. Gerhartz HH, Engelhard M, Meusers P, Brittinger G, Wilmanns W, Schlimok G, Mueller P, Huhn D, Musch R, Siegert W, Gerhartz D, Hartlapp J, Thiel E, Huber C, Peschl C, Spann W, Emmerich B, Schadek C, Westerhausen M, Pees HW, Radtke H, Engert A, Terhardt E, Schick H, Binder T, Fuchs R, Hasford J, Brandmaier R, Stern AC, Tiemann M, Lennert K (1993) Randomized, double-blind, placebo-controlled, phase III study of recombinant human granulocyte-macrophage colony-stimulating factor as adjunct to induction treatment of high-grade malignant non-Hodgkin's lymphomas. Blood 82:2329–2339
6. Crawford J, Ozer H, Stoller R, Johnson D, Lyman G, Dabbara I, Kris M, Grous J, Picozzi V, Rausch G, Smith R, Gradishar W, Yahanda A, Vicent M, Stewart M, Glaspy J (1988) Reduction by granulocyte colony-stimulating factor of fever and neutropenia induced by chemotherapy in patients with small cell lung cancer. N Engl J Med 325:164–170
7. Ardizzoni A, Venturini M, Sertoli MR, Giannessi PG, Brema F, Danova M, Testore F, Mariani GL, Pennucci MC, Queirolo P, Silvestro S, Bruzzi P, Lionetto R, Latini F, Rosso R (1994) Granulocyte-macrophage colony-stimulating factor (GM-CSF) allows acceleration and dose-intensity increase of CEF chemotherapy. A randomised study in patients with advanced stage breast cancer. Br J Cancer 69:385–391
8. Del Mastro L, Garrone O, Sertoli MR, Canavese G, Catturich A, Guenzi M, Rosso R, Venturini M (1994) A pilot study of accelerated cyclophosphamide, epirubicin and 5-fluorouracil plus granulocyte-colony stimulating factor as adjuvant therapy in early breast cancer. Eur J CAncer 30A:606–610
9. Case DC Jr, Bukowski RM, Carey RW, Fishkin EH, Henry DH, Jacobson RJ, Jones SE, Keller AM, Kugler JW, Nichols CR, Salmon SE, Silver RT, Storniolo AM, Wampler GL, Dooley CM, Larholt KM, Nelson RA, Abels RI (1993) Recombinant human erythropoietin therapy for anemic cancer patients on combination chemotherapy. J Natl Cancer Inst 85:801–806
10. Kurzrock R, Talpaz M, Gutterman JU (1992) Very low doses of GM-CSF administered alone or with erythropoietin in aplastic anaemia. Am J Med 93:41–48
11. Pedrazzini A (1993) Erythropoietin and GM-CSF following autologous bone marrow transplantation. Eur J Cancer 29A [Suppl 2] :S15–S17
12. Krumwieh D, Siebold B, Weinmann E, Weinmann E, Seiler FR (1991) Combined effect of granulocyte-macrophage colony stimulating factor with erythropoietin in subhuman primates. Am J Clin Oncol 14 [Suppl 1] :S1–S4
13. American Joint Committee on Cancer (1992) Breast manual for staging of cancer, 4th edn. Lippincott, Philadelphia, pp 149–154

14. Miller AB, Hoogstraten AB, Staquet M, Winkler A (1981) Reporting results of cancer treatment. Cancer 47: 207–214
15. Lieschke GJ, Cebon J, Morstyn G (1989) Characterization of the clinical effects after the first dose of bacterially synthesized recombinant human granulocyte-macrophage colony stimulating factor. Blood 74: 2634–2643
16. Ardizzoni A, Venturini M, Crinò L, Sertoli MR, Bruzzi P, Pennucci MC, Mariani GL, Garrone O, Bracarda S, Rosso R, Van Zandwijk N (1993) High dose-intensity chemotherapy, with accelerated cyclophosphamide-doxorubicin-etoposide and granulocyte-macrophage colony stimulating factor, in the treatment of small cell lung cancer. Eur J Cancer 29A: 687–692
17. Henderson IC, Harris JR, Kinne DW, Hellman S (1989) Cancer of the breast. In: De Vita VT Jr, Hellman S, Rosemberg SA (eds) Cancer: principles and practice of oncology, 3rd edn. Lippincott, Philadeplphia, pp 1197–1268
18. O'Shaughnessy JA, Denicoff AM, Venzon DJ, Danforth D, Pierce LJ, Frame JN, Bastian A, Ghosh B, Goldspiel B, Miller L, Dorr FA, Keegan P, Ben-Baruch N, Mrose H, Noone M, Cowan KH (1994) A dose intensity study of FLAC (5-fluorouracil, leucovorin, doxorubicin, cyclophosphamide) chemotherapy and *Escherichia coli*-derived granulocyte-macrophage colony-stimulating factor (GM-CSF) in advanced breast cancer patients. Ann Oncol 5: 709–716
19. Mamounas EP, Anderson S, Wickerham DL, Clark R, Stoller R, Hamm JT, Stewart JA, Bear HD, Glass AG, Bornstein R, Fisher B (1994) The efficacy of recombinant human granulocyte colony-stimulating factor and recombinant human granulocyte macrophage colony-stimulating factor in permitting the administration of higher doses of cyclophosphamide in a doxorubicin-cyclophosphamide combination. Am J Clin Oncol 17: 374–381
20. Hoekman K, Wagstaff J, Van Groeningen CJ, Vermorken JB, Boven E, Pinedo HM (1991) Effects of recombinant human granulocyte-macrophage colony stimulating factor on myelosuppression induced by multiple cycles of high-dose chemotherapy in patients with advanced breast cancer. J Natl Cancer Inst 83: 1546–1553
21. Bonadonna G, Gianni L, Santoro A, Bonfante V, Bidoli P, Casali P, Demicheli R, Valagussa P (1993) Drugs ten years later: epirubicin. Ann Oncol 4: 351–369
22. Bronchud MH, Howell A, Crowther D, Hopwood P, Souza L, Dexter TM (1989) The use of granulocyte colony-stimulating factor to increase the intensity of treatment with doxorubicin in patients with advanced breast and ovarian cancer. Br J Cancer 60: 121–125
23. Hamm J, Schiller JH, Cuffie C, Oken M, Fisher RI, Shepherd F, Kaiser G (1994) Dose-ranging study of recombinant human granulocyte-macrophage colony-stimulating factor in small cell lung carcinoma. J Clin Oncol 12: 2667–2676