no more than 5%-7% greater than the rate observed before the increase started.

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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

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In this study, 18 patients with advanced breast cancer were treated with multiple cycles of doxorubicin (75 or 90 mg/m²) plus cyclophosphamide (750 or 1000 mg/m²) every 21 days. Granulocyte-macrophage colony-stimulating factor (GM-CSF) (250 µg/m² per day) was administered by continuous infusion during 10 days (days 2-12), starting in the first or second cycle of chemotherapy. Sixteen (89%) of 18 patients (95% confidence interval, 65%-99%) achieved an objective remission, five (28%) of which were complete. The median duration of response was 7 months. When GM-CSF was used for the first time, it had an effect on the kinetics of all blood cells, including neutrophils, lymphocytes, thrombocytes, and reticulocytes. However, in subsequent cycles of chemotherapy, the stimulatory effect of GM-CSF on hematopoiesis was substantially diminished. World Health Organization grade 3 and 4 neutropenia and thrombocytopenia necessitated dose reductions of doxorubicin and

cyclophosphamide from cycle 2 onward in all patients treated with the highest dose. Side effects of GM-CSF included fever, general weakness, and hypotension. These toxic effects mimicked sepsis, and hospital admission for treatment with intravenous antibiotics was required for 73 days in 61 cycles of chemotherapy that included GM-CSF. Dose-intensive chemotherapy produced a high response rate in patients with advanced breast cancer. However, GM-CSF administered from day 2 to day 12 at a dose of 250 µg/m²

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per day by continuous infusion did not adequately ameliorate the myelosuppression induced by repeated cycles of dose-intensive chemotherapy. [J Natl Cancer Inst 83:1546-1553, 1991]

Increasing the dose or frequency of administration of cytostatic drugs might be expected to lead to an improved therapeutic outcome of cancer treatment (1). This result seems to be true for doxorubicin (2,3) and for alkylating agents (4) in the treatment of breast cancer.

Increasing dose intensity is, however, usually accompanied by a concurrent increase in toxic effects, which generally limit the degree of dose escalation that may be achieved in practice. One of the major dose-limiting toxic effects is myelosuppression, which, if severe, can lead to life-threatening infection or hemorrhage. Bone marrow transplantation is a possible means of overcoming the problem of myelosuppression, but this procedure cannot be performed more than once or twice. The availability of hematopoietic growth factors offers another possibility of using high-dose chemotherapy more safely.

These hematopoietic growth factors constitute a family of glycoproteins which support the proliferation and differentiation of hematopoietic progenitors of various lineages (5). Moreover, they stimulate the biological activity of their mature offspring. Among the hematopoietic growth factors, granulocyte-macrophage colony-stimulating factor (GM-CSF) has been shown to stimulate the growth of colonies of neutrophils, eosinophils, monocytes-macrophages, and, to a lesser extent, cells of the erythroid and megakaryocyte lineages (6). Phase I studies have defined a dose of GM-CSF which, with acceptable toxicity, increases the rate of recovery of the white blood cell count following chemotherapy (7).

The aim of our study was to determine the maximum doses of the chemotherapeutic drugs, given in multiple cycles together with GM-CSF, which could be safely administered to patients with advanced breast cancer. Doxorubicin and cyclophosphamide were selected as being two of the most active agents in the treatment of this disease. GM-CSF was given by continuous infusion at a dose of 250 $\mu\text{g}/\text{m}^2$ per day during 10 consecutive days. This schedule of administration was chosen because it has been demonstrated that a continuous and minimal (≥ 1 ng/mL) concentration of GM-CSF is required for the optimum stimulation of hematopoietic precursors (8).

Patients and Methods

Patient Selection

Eligible patients included women between 18 and 70 years of age with histologically verified locally advanced (not curable by surgery) or metastatic breast cancer and a performance status of 2 or less (World Health Organization [WHO] or Eastern Cooperative Oncology Group).

Evidence of adequate bone marrow function (white blood cell count $\geq 4.0 \times 10^9/\text{L}$ and platelet count $\geq 100 \times 10^9/\text{L}$), renal function (creatinine level ≤ 150 $\mu\text{mol}/\text{L}$), and hepatic function (bilirubin level ≤ 25 $\mu\text{mol}/\text{L}$) was required. Active infection, a history of cardiovascular disease, and evidence of central ner-

vous system metastases were exclusion criteria. The disease had to be clinically or radiologically evaluable or measurable (9). All patients gave written informed consent, and the protocol was approved by the ethical and scientific review committees of the Free University Hospital, Amsterdam.

Treatment

Patients were treated with doxorubicin plus cyclophosphamide by intravenous bolus injection every 21 days until either dose-limiting toxic effects occurred or the patient had received a cumulative dose of 550 mg/m^2 doxorubicin. The starting doses were 75 mg/m^2 for doxorubicin and 750 mg/m^2 for cyclophosphamide. In subsequent groups of patients, the dose of doxorubicin was increased by 15 mg/m^2 and that of cyclophosphamide by 250 mg/m^2 . Initially, chemotherapy was given without GM-CSF. When WHO grade 4 toxic effects occurred (white blood cell count $< 1.0 \times 10^9/\text{L}$ and/or platelet count $< 25 \times 10^9/\text{L}$), GM-CSF was added in the next cycle. If a similar degree of hematologic toxic effects was observed in the subsequent cycle of chemotherapy with GM-CSF, then the next group of patients received the same chemotherapeutic doses, but with GM-CSF administered in the first cycle.

A maximum tolerated chemotherapeutic dose was defined as a dose which caused WHO grade 4 toxic effects of greater than 4 days' duration, despite the use of GM-CSF from the first cycle onward in three of five patients. Doses were reduced when, in a preceding cycle with GM-CSF, stomatitis or hematologic toxic effects (WHO grade 3 or 4) occurred which continued for more than 4 days. The dose of doxorubicin was then reduced by 7.5 mg/m^2 , and the dose of cyclophosphamide was reduced by 125 mg/m^2 in the next cycle of chemotherapy. The dose-reduction steps were small because we intended to maximize the amount of drug given to each patient. Treatment was delayed for 1 week if the leukocyte count was less than $3.0 \times 10^9/\text{L}$ and/or the thrombocyte count was less than $100 \times 10^9/\text{L}$. Erythrocyte transfusions were given when the hemoglobin level decreased to less than 6.0 mmol/L, and prophylactic platelet transfusions were given when the platelet count was less than $10 \times 10^9/\text{L}$. All patients used antiseptic mouthwashes prophylactically; however, no prophylactic antibiotics were given. Patients left the hospital on day 2 and were readmitted on day 21.

Granulocyte-Macrophage Colony-Stimulating Factor

The *Escherichia coli*-derived nonglycosylated recombinant human GM-CSF was supplied free of charge by Behringwerke A.G. (Marburg, Federal Republic of Germany). It was provided as a lyophilized powder and was reconstituted with sterile water prior to the start of the continuous infusion. GM-CSF was given at a dose of 250 $\mu\text{g}/\text{m}^2$ per day via a Port-A-Cath (Pharmacia Deltec, St. Paul, Minn.) using a CADD-1 portable pump (Pharmacia Deltec). The infusion was started 24 hours after chemotherapy began and was continued for 10 days. The 50-mL medication cassette was refilled after 5 days, the total volume of the infusion being 100 mL.

Clinical and Laboratory Monitoring

Complete hematological screening, including differential cell counts, was performed every 2-3 days, and biochemical analysis

was carried out weekly. The patients were examined once each week, and their symptoms and vital signs were recorded. Patients were asked to record their axillary temperature twice daily and to note any specific complaints. Tumor response was evaluated after each cycle of chemotherapy, according to International Union Against Cancer criteria (9). The left ventricular ejection fraction was measured every two cycles and after discontinuation of chemotherapy. If the left ventricular ejection fraction fell to less than 50%, then the patient was withdrawn from the study and further treatment was given at the discretion of the treating physician. In six patients, the existence of GM-CSF antibodies was monitored by using an enzyme-linked immunosorbent assay technique.

Statistical Analysis

The statistical significance of differences was evaluated using the two-sided unpaired Student's *t* test.

Results

Eighteen patients participated in the study, and their characteristics and responses to therapy are documented in Table 1. All patients had measurable disease and were assessable for response. Twelve patients were not assessable for remission duration because of continuation of the treatment by another modality. Eleven patients had been treated with irradiation to a total dose of 50-60 Gy after their mastectomy. Eight patients had locally advanced disease at study entry and received local treatment after responding to chemotherapy. Three patients stopped treatment because of toxic effects and were subsequently given standard chemotherapy.

Three successive groups of patients were available for the analysis of the effects of GM-CSF. Group A consisted of five patients treated with 75 mg/m² doxorubicin plus 750 mg/m² cyclophosphamide; GM-CSF was started after grade 4 toxic effects in a preceding cycle of chemotherapy. Subsequently, six patients (group B) were treated with a starting dose of 90 mg/m² doxorubicin plus 1000 mg/m² cyclophosphamide; GM-CSF was begun in cycle 2. Despite the use of GM-CSF in the second cycle, WHO grade 3 or 4 neutropenia and thrombocytopenia occurred in all patients in this cycle. Therefore, the next group of seven patients (group C) received the same chemotherapeutic dose as group B but started with GM-CSF in the first cycle.

Effect of GM-CSF on Myelosuppression Induced by 75 mg/m² Doxorubicin Plus 750 mg/m² Cyclophosphamide

The treatment of five patients with 75 mg/m² doxorubicin plus 750 mg/m² cyclophosphamide without GM-CSF produced severe myelosuppression (Table 2). The absolute neutrophil count fell to a mean nadir of $0.17 \times 10^9/L$ (range, $0-0.28 \times 10^9/L$). The median duration of neutropenia ($\leq 0.5 \times 10^9/L$) was 6 days (range, 5-9 days), and recovery of neutrophils (to $\geq 2.0 \times 10^9/L$) did not occur until day 21. Significant thrombocytopenia did not occur during this first cycle of chemotherapy.

The administration of GM-CSF in the second cycle of chemotherapy produced a marked improvement in the hematological profile. The absolute neutrophil count nadir was $0.72 \times 10^9/L$, and none of the patients developed neutrophil counts less than or equal to $0.5 \times 10^9/L$. The absolute neutrophil count was $2.0 \times 10^9/L$ or higher by day 12 (range, 11-15 days). The mean platelet count nadir fell to $101 \times 10^9/L$.

Table 1. Patient characteristics and treatment results*

Group and patient No.	Stage	Prior therapy	Cycles without GM-CSF	Cycles with GM-CSF	Response	Duration of response, mo	Survival, mo
Group A							
1	MD	RT, HT, CT	1	1	PR	NA (a)	32
2	MD	RT	1	2	SD	NA (a)	21
3	LD		3	1	PR	NA (b)	30+
4	MD	RT, HT	1	3	PR	NA (a)	16
5	LD	HT	1	1	PR	6	14
Group B							
1	LD		1	2	PR	NA (b)	16
2	LD		1	2	PR	NA (b)	20+
3	MD	RT	1	4	CR	9	21+
4	MD	RT, HT	1	5	SD	7	16+
5	MD	RT	1	5	PR	8	11
6	MD	RT, HT	1	1	PR	NA (a)	23+
Group C							
1	LD	RT, HT	0	4	CR	NA (b)†	16+
2	LD		0	4	CR	NA (b)†	17+
3	MD	RT, HT	0	5	PR	12‡	14
4	MD	RT	0	5	CR	6	10
5	MD	RT	0	4	PR	NA (a)	9+
6	LD		0	6	PR	NA (b)	9+
7	LD		0	6	CR	NA (b)	8+

*Abbreviations: MD = metastatic disease, LD = locally advanced disease, RT = radiotherapy, HT = hormone therapy, CT = chemotherapy, CR = complete remission, PR = partial remission, SD = stable disease, NA (a) = not assessable because of further treatment with chemotherapy, and NA (b) = not assessable because of further therapy with surgery and/or radiotherapy to the primary lesion.

†Recurrence as carcinomatous meningitis after 7 mo.

‡Recurrence as carcinomatous meningitis after 12 mo.

Table 2. Count nadirs (means) and duration (medians) of neutropenia and thrombocytopenia with sequential cycles of chemotherapy

Group and cycle No.	Mean doses of doxorubicin/ cyclophosphamide, mg/m ²	No. of patients	Absolute neutrophil count			Platelet count		
			Nadir, × 10 ⁹ /L	Duration, d		Nadir, × 10 ⁹ /L	Duration, d	
				<1.0 × 10 ⁹ /L	<0.5 × 10 ⁹ /L		<100 × 10 ⁹ /L	<50 × 10 ⁹ /L
Group A								
1	75.0/750	5	0.17	9	6	122	0	0
2*	75.0/750	5	0.72	3	0	101	0	0
3*	75.0/750	2	0.37	9	3	64	5	0
Group B								
1	90.0/1000	6	0.08	7	7	60	2	0
2*	90.0/1000	6	0.13	7	4	34	5	2
3*	82.5/875	4	0.10	9	4	24	9	5
4*	82.5/875	3	0.08	11	7	11	14	11
5*	75.0/750	3	0.15	11	7	17	11	5
6*	75.0/750	2	0.02	9	7	9	18	11
Group C								
1*	90.0/1000	7	0.08	5	4	74	3	0
2*	82.5/875	7	0.28	9	4	56	5	0
3*	82.5/875	7	0.09	9	7	17	9	7
4*	75.0/750	7	0.18	9	4	37	7	3
5*	75.0/750	3	0.12	10	6	15	11	7

*Chemotherapy cycles with GM-CSF.

By the third cycle (the second cycle with GM-CSF), the beneficial effect of GM-CSF had already diminished, and patients again developed WHO grade 4 hematologic toxic effects, with recovery to a neutrophil count of $2.0 \times 10^9/L$ or higher not occurring until day 18. Cumulative platelet toxicity was evidenced by a further fall in the mean platelet count nadir ($64 \times 10^9/L$) with recovery to $100 \times 10^9/L$ or higher not occurring until day 14.

Comparison of Myelosuppression Induced by a First Cycle of High-Dose Chemotherapy in the Absence or Presence of GM-CSF

The hematological parameters of the first cycle of patients in group B and C are compared in Fig. 1 and Table 2.

After the start of GM-CSF, the absolute neutrophil count rose rapidly to a mean peak of $17.1 \times 10^9/L$ (range, 8.9 – $24.9 \times 10^9/L$) on day 4 of the chemotherapeutic cycle. It then fell rapidly to a nadir on day 9, but recovery began immediately, with the number of days at the less than or equal to $0.5 \times 10^9/L$ level being 3 days less in those patients receiving GM-CSF (Table 2). Recovery to a value of $2.0 \times 10^9/L$ or higher occurred on day 16, compared with day 18 in those patients not given GM-CSF. During the recovery phase in response to GM-CSF, there was a prominent increase in the number of immature granulocytes in the peripheral blood, which was most marked on day 14 (Fig. 1). With subsequent cycles of chemotherapy together with GM-CSF, the extent of this response was markedly and progressively diminished (results not shown).

GM-CSF induced an eosinophilia, which was restricted to the time of GM-CSF administration. This eosinophilia occurred with a similar temporal pattern during almost all cycles of chemotherapy given together with GM-CSF.

In the first cycle with GM-CSF, the severity of the lymphocyte count nadir and duration of lymphopenia of less than or equal to $0.5 \times 10^9/L$ increased, compared with those seen in the cycle without GM-CSF. This phenomenon was apparent in all

three groups of patients. The lymphopenia was followed by a rebound lymphocytosis (Fig. 1).

High-dose chemotherapy induced a fall in the erythrocyte mass and their precursors (as judged by the reticulocyte count).

Fig. 1. Peripheral blood counts of two groups of patients with locally advanced or metastatic breast cancer treated for the first time with 90 mg/m² doxorubicin and 1000 mg/m² cyclophosphamide, either with or without GM-CSF. Values are given as means ($\times 10^9/L$) and SDs, except for those for reticulocytes, which are given as $\times 10^6/L$.

The reticulocyte count rapidly declined to zero without and with GM-CSF. The mean reticulocyte counts were significantly higher ($P<.05$) on days 12, 14, 16, and 18 in patients given GM-CSF during the first cycle of chemotherapy (Fig. 1).

High-dose doxorubicin plus high-dose cyclophosphamide also induced a thrombocytopenia, which was already evident in the second cycle. In the presence of GM-CSF, the platelet count nadir occurred 3 days earlier but was as deep as that seen in patients not given GM-CSF. The mean platelet counts were significantly higher ($P<.05$) on days 12, 14, 16, and 18 in patients given GM-CSF (Fig. 1). The duration of thrombocytopenia below $100 \times 10^9/L$ was, however, similar in the two groups (Table 2).

Finally, it was evident that GM-CSF was less effective in this high-dose chemotherapy group than in group A, which was treated with 75 mg/m^2 doxorubicin and 750 mg/m^2 cyclophosphamide, in view of the neutrophil count nadir and the duration of neutropenia (Table 2).

Effect of GM-CSF on Myelosuppression Induced by Repeated Cycles of High-Dose Chemotherapy

Treatment with 90 mg/m^2 doxorubicin plus 1000 mg/m^2 cyclophosphamide resulted in a deep neutropenia with a mean absolute neutrophil count nadir of $0.08 \times 10^9/L$ in the absence or presence of GM-CSF. In subsequent cycles of chemotherapy, doses were reduced because of grade 4 leukopenia and/or thrombocytopenia lasting more than 4 days (Table 2). Chemotherapy was delayed in only two patients because they had a platelet count less than $100 \times 10^9/L$ during cycles 4 and 5, respectively. As can be seen from Fig. 2, A and B, there was a marked slowing in the rate of recovery of neutrophils after the first administration of GM-CSF. In group C, the absolute neutrophil count recovered to a value of $2.0 \times 10^9/L$ or higher on day 16 in the first course of chemotherapy with GM-CSF, but recovery took place on day 18 in cycle 2 and on day 21 in cycle 5.

Sequential cycles of chemotherapy resulted in a thrombocytopenia of increasing severity (Table 2) and prolonged duration (Fig. 2, C and D). Prophylactic platelet transfusions, administered because the platelet count was less than $10 \times 10^9/L$, were required in 11 (35%) of 31 cycles given from cycle 3 onward, despite mild chemotherapeutic dose reductions. The mean time for the platelet count to recover to $100 \times 10^9/L$ or higher increased from 3 days in cycle 1 to 11 days in cycle 5 (Table 2). Cumulative myelosuppression was also evidenced by the increasing requirement for erythrocyte transfusions as chemotherapy continued. Blood transfusions were necessary in 33 (62%) of 53 cycles at the higher chemotherapeutic dose level.

In six patients, in whom the stimulatory effects of GM-CSF decreased during successive cycles of chemotherapy, antibodies against GM-CSF were monitored. Such antibodies could not be detected in any of these patients.

Complications of High-Dose Chemotherapy Plus GM-CSF

The occurrence of neutropenic fever (axillary temperature $>38.0^\circ\text{C}$ and neutropenia $<0.5 \times 10^9/L$) was sometimes a reason for hospital admission to administer intravenous antibiotics. In-

Fig. 2. Patterns of recovery of the neutrophil and platelet counts of patients with locally advanced or metastatic breast cancer treated with multiple cycles of high-dose doxorubicin plus cyclophosphamide. GM-CSF was started either in the first (group C) or the second (group B) cycle of chemotherapy. Values are given as means ($\times 10^9/L$) and SDs (first and fifth cycles only). Panel A: neutrophil counts in group B. Panel B: neutrophil counts in group C. Panel C: platelet counts in group B. Panel D: platelet counts in group C.

travenous antibiotics were started five times (38%) in 13 cycles without GM-CSF and nine times (15%) in 61 cycles with GM-CSF. The duration of antibiotic treatment was 31 days (13.4%) during 231 days that patients not receiving GM-CSF were at risk and 73 days (5.6%) during 1295 days that patients receiving GM-CSF were at risk. Infections were proven by bacterial culture in two (18%) of 11 cycles of chemotherapy without GM-CSF and seven cycles (11%) of 61 cycles with GM-CSF.

In the majority of cases, the fever seemed to be caused by GM-CSF. The typical GM-CSF-induced fever is transient, occurs mostly in the evenings, and is not accompanied by symptoms suggestive of infection. Indeed, our ability to discern whether fever was due to infection or GM-CSF improved as the study progressed. This improvement is illustrated by the fact that, in group B, antibiotics were started seven times in 19 cycles with GM-CSF, compared with two times in 34 cycles with GM-CSF in the subsequently treated group C.

Stomatitis of grade 1 severity was almost universal. Grade 2/3 stomatitis was observed in 10 of 53 cycles of the higher chemotherapeutic dose level and in one of 13 cycles at the lower level. No cutaneous toxic effects were observed in these patients.

The mean arterial pressure was monitored during the 1st days of administration of GM-CSF. GM-CSF caused a decrease of 10-15 mm Hg in the mean arterial pressure of almost every

patient. This phenomenon was not observed following the administration of chemotherapeutic drug alone. This mild reduction in blood pressure was present in all courses with GM-CSF, but it never necessitated additional supportive care. Renal function did not deteriorate during GM-CSF administration.

Treatment with high-dose chemotherapy was associated with general weakness of grade 1/2 severity. The administration of GM-CSF exacerbated this problem, with grade 2 weakness ($\leq 50\%$ of normal activity) occurring in 44 of 61 cycles with GM-CSF. These symptoms were so severe in three patients that GM-CSF had to be stopped.

In 18 of 61 GM-CSF cycles, reversible abnormalities of liver enzymes (especially high alkaline phosphatase and γ -glutamyl-transferase) of grade 2/3 severity were observed. The abnormalities recovered rapidly after discontinuation of GM-CSF. Chemotherapy caused a small, but nonsignificant, fall in mean serum albumin values (from 34.8 to 31.6 g/L in cycle 1 of group B). The administration of GM-CSF enhanced this decrease, such that the mean values fell from 34.5 to 23.6 g/L in cycle 2 of group B ($P < .05$). In group C, where GM-CSF was given in the first cycle, the mean serum albumin also fell significantly ($P < .05$) from 35.7 to 26.4 g/L.

Thrombosis of the subclavian vein communicating with the Port-A-Cath occurred in three of 18 patients (in zero of 13 cycles without GM-CSF and in three of 61 cycles with GM-CSF).

One patient developed a serious cardiomyopathy after six cycles of chemotherapy. This patient had been previously treated with radiotherapy to the left thorax and received a total of 495 mg/m² doxorubicin. The left ventricular ejection fraction was normal (62%) before the sixth course but was low (24%) 3 weeks after the end of the sixth cycle. This decline was accompanied by a serious forward cardiac failure. In two other patients given a cumulative dose of 300 and 435 mg/m² doxorubicin after four courses, treatment was stopped because the left ventricular ejection fraction decreased to less than 50%. These patients showed no clinical evidence of heart failure. In the other 15 patients, no cardiac problems became manifest.

Antitumor Effects

Of the first five patients receiving 75 mg/m² doxorubicin plus 750 mg/m² cyclophosphamide, four patients showed an objective clinical response (four partial responses and one stable disease). Of the 13 patients initially treated with 90 mg/m² doxorubicin plus 1000 mg/m² cyclophosphamide, 12 patients responded (five complete responses, seven partial responses, and one stable disease). The overall response rate in the 18 patients was therefore 89% (95% confidence interval, 65%-99%). The median time to disease progression in the group with metastatic breast cancer was 7 months (range, 6-12 months). In three patients in group C, the disease recurred as carcinomatous meningitis and/or brain metastases without evidence of disease outside the central nervous system. The survival of the whole group of patients varied from 8 to 32 months, with a median of 16 months. Ten patients are currently alive (as of August 1991), and four patients treated for locally advanced breast cancer still have no evidence of metastatic disease.

Discussion

The aim of this study was to define the maximum tolerated doses of doxorubicin and cyclophosphamide that could be administered together with a fixed dose of GM-CSF in patients with advanced breast cancer. Doxorubicin (90 mg/m²), together with cyclophosphamide (1000 mg/m²), produced a marked degree of myelosuppression, even when the GM-CSF was administered from the first cycle of chemotherapy onward. Reductions in doses were therefore necessary, and the optimum doses of these two agents for use in repeated cycles are probably somewhat less than 90 mg/m² and 1000 mg/m², respectively. The maximum dose administered in this study does, however, represent an increase of 80% for doxorubicin and 70% for cyclophosphamide, measured in milligrams per square meter per week, over standard dose programs (50 mg/m² doxorubicin and 600 mg/m² cyclophosphamide every 3 weeks).

Other studies have attempted to increase dose intensity by not only raising the dose of the chemotherapeutic agents but also increasing the frequency of administration (10). An increased dose intensity was made possible by the more rapid recovery of the neutrophil counts, which occurred as a consequence of the administration of G-CSF, allowing doxorubicin to be given every 2 weeks instead of every 3 weeks. In our patients, who were given doxorubicin together with cyclophosphamide, it would not have been possible to administer multiple cycles of these drugs on a 14-day schedule. The slower recovery in our patients could have been due to the combination of cytotoxic agents that we used or to the fact that we used GM-CSF instead of G-CSF.

During the first cycle of chemotherapy, GM-CSF affected the pattern of neutrophil decline and recovery which has been well described by others (11). In addition, it also had small, but significant, effects on the rate of recovery of platelets and erythrocytes (Fig. 1). These findings are consistent with in vitro data indicating that GM-CSF affects cell lineages other than granulocytes and monocytes-macrophages (12,13).

We observed a reduction in the efficacy of GM-CSF in relation to both the dose of chemotherapeutic drug and the number of cycles administered. As can be seen from Fig. 2, the rate of neutrophil recovery slowed down in the successive cycles in which GM-CSF was administered. This decline was especially pronounced on the second occasions that GM-CSF was administered (group B, cycle 3; group C, cycle 2), which is all the more remarkable since this reduced efficacy of GM-CSF coincided with the first dose reduction in both of these groups of patients. The subsequent decrease in the rate of neutrophil recovery in successive cycles was less marked. A similar phenomenon was observed in the patterns of platelet recovery (Fig. 2). Again, there was a marked slowing of the rate of platelet recovery on the second occasion that GM-CSF was administered. This observation is a clear indication that the beneficial effects of this hematopoietic growth factor are substantially less after repeated use.

The rapid reduction in efficacy of GM-CSF after its first administration requires explanation. It is not completely explained by cumulative stem cell damage due to the chemotherapy, because this damage might be expected to induce a more gradual reduction in the rate and magnitude of recovery. Another pos-

sibility might be that recombinant nonglycosylated GM-CSF is antigenic and that antibodies generated in vivo were antagonizing its effects. However, to date, such neutralizing antibodies have not been detected in patients receiving GM-CSF (14). An alternative explanation might be that the application of supraphysiological doses of GM-CSF leads to the induction of negative feedback loops capable of inhibiting hematopoiesis. Natural inhibitors of hematopoiesis have been described (15,16), and one of these (i.e., tumor necrosis factor) has been shown to be produced in vivo during GM-CSF administration (17). In addition, it has been demonstrated that marrow granulocyte-macrophage progenitors from patients treated with GM-CSF displayed increased sensitivity to the growth-inhibitory effects of tumor necrosis factor- α (18). A more complete understanding of the interactions between stimulators and inhibitors of hematopoiesis may allow us to manipulate these processes more efficiently.

Despite the continued use of GM-CSF, a progressive and severe thrombocytopenia developed when repetitive cycles of high-dose chemotherapy were administered (Fig. 2). Thrombocytopenia became dose limiting after three or four cycles of chemotherapy. The requirement for erythrocyte transfusions also increased as chemotherapy continued. Other hematopoietic growth factors, such as interleukin-1, interleukin-3, or interleukin-6, which act on a broader spectrum of cell lineages and at an earlier stage in their differentiation pathways, may be capable of reducing myelosuppression if used alone (19-21) or in combination with later acting factors (22-24).

In the first cycle with GM-CSF, the peripheral blood lymphocyte counts fell to levels lower than those seen following chemotherapy alone (Fig. 1). This phenomenon was not due to a direct effect of GM-CSF on lymphocytes, because these cells do not express GM-CSF receptors. GM-CSF probably induces the secondary production of other cytokines (25), which cause a perturbation of the normal pattern and tempo of lymphocyte recirculation.

More patients developed subclavian vein thromboses than we had expected. This coagulopathy might have been caused by secondary cytokines induced by GM-CSF. Among them, tumor necrosis factor is a potent activator of endothelial cells and is, therefore, able to initiate the coagulation cascade (26). Further investigation of the coagulation status of patients receiving GM-CSF is required.

The toxicity of GM-CSF caused problems in treating these patients. GM-CSF caused pyrexia, malaise, and a mild reduction in mean arterial pressure. In neutropenic patients, these conditions can closely mimic sepsis, and as a consequence a substantial proportion of our patients were admitted to the hospital and given intravenous antibiotics. The actual incidence of documented infections was, however, low, and as the study continued we became more efficient at differentiating between GM-CSF toxicity and infection. The frequency of admission for treatment with antibiotics was therefore lower in the last cohort of patients entered in the study. It was, however, a matter of some concern to allow a febrile neutropenic patient to stay at home without antibiotic support.

The administration of GM-CSF was also associated with a significant decrease in serum albumin. This decrease may have

been due to capillary leakage and/or may be indicative of an acute-phase response to GM-CSF or to secondary cytokines induced by GM-CSF.

In three of 18 patients treated in this study, abnormal cardiac function developed after relatively low cumulative doses of doxorubicin. This observation raises the possibility that the pharmacokinetics of doxorubicin may be different when it is given in higher doses and in combination with GM-CSF (27). This aspect is of considerable clinical relevance and, therefore, requires further study.

The increase in dose intensity used in this study did produce an increase in the response rate similar to that observed in other dose-intensive programs (2-4,10). The duration of remissions in our study was, however, disappointingly short. The impact that these dose-intensive protocols have on the survival of patients with advanced breast cancer remains to be established.

In this study, GM-CSF during its first administration after high-dose chemotherapy substantially reduced the period of neutropenia. This effect was much diminished with subsequent cycles, and thrombocytopenia became dose limiting. These data indicate that high-dose chemotherapy might increase the probability of response in patients with advanced breast cancer but that other single hematopoietic growth factors or combinations of hematopoietic growth factors will be required for an optimum and continuing stimulation of recovery of neutrophils and platelets after dose-intensive chemotherapy.

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Comparison of Tritiated Estradiol and Tamoxifen Aziridine for Measurement of Estrogen Receptors in Human Breast Cancer Cytosols

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We examined the estrogen receptor measurement in 265 human breast cancer cytosols by using a specific method based on [^3H]tamoxifen aziridine labeling, sequential immunoadsorption with an antiestrogen receptor monoclonal antibody (H-222), sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and autoradiography. These new tools of molecular endocrinology revealed an impressive estrogen receptor molecular polymorphism. Given the recent finding of a similar estrogen receptor polymorphism at the messenger RNA level by several laboratories, it is tempting to speculate about its possible biological significance. To gain insight into the potential clinical relevance of this polymorphism in terms of breast cancer hormone dependence, we compared the 265 cytosols for their [^3H]tamoxifen aziridine- and [^3H]estradiol-binding capacities using the above-mentioned method and the conventional dextran-coated charcoal assay. We failed to identify a specific [^3H]tamoxifen aziridine electrophoretic pattern with respect to the tumor estrogen receptor content as measured by the dextran-coated charcoal assay. However, an excellent correlation overall was found between the intensities of both labeling methods. Some tumors were positive for only one of these two ligands. It will be clinically important to see whether the

tumors positive for [^3H]tamoxifen aziridine only correspond to the small subset of tumors (10%) which respond to tamoxifen treatment despite very low estrogen receptor levels, as measured by the dextran-coated charcoal technique. [*J Natl Cancer Inst* 83:1553-1559, 1991]

As clinicians, we continue to rely heavily on knowledge of the estrogen receptor status in the management of individual patients with breast cancer. Indeed, estrogen receptor concentrations remain among the most important factors for predicting the patient's prognosis as well as response to endocrine therapy (1). Tremendous advances in our understanding of the basic structure and function of the estrogen receptor have been made in the last 5 years (2-4). The estrogen receptor protein has been shown

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