Sequential In Vivo Treatment With Two Recombinant Human Hematopoietic Growth Factors (interleukin-3 and granulocyte-macrophage colony-stimulating factor) as a New Therapeutic Modality to Stimulate Hematopoiesis: Results of a Phase I Study

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In a phase I study, the sequentially administered combination of recombinant human interleukin-3 (rhIL-3) and rhGM-CSF was compared with treatment with rhIL-3 alone in 15 patients with advanced tumors but normal hematopoiesis. Patients were initially treated with rhIL-3 for 15 days. After a treatment-free interval, the patients received a second 5-day cycle of rhIL-3 at an identical dosage, immediately followed by a 10-day course of rhGM-CSF, to assess the toxicity and biologic effects of this sequential rhIL-3/rhGM-CSF combination. rhIL-3 doses tested were 125, and 250 μg/m², whereas rhGM-CSF was administered at a daily dosage of 250 μg/m². Both cytokines were administered by subcutaneous (SC) bolus injection. rhIL-3/rhGM-CSF treatment was more effective than rhIL-3 but equally effective to each other in increasing peripheral leukocyte counts, especially neutrophilic and eosinophilic granulocyte counts. In contrast, both modes of cytokine therapy raised the platelet counts to the same degree. rhIL-3/GM-CSF treatment was more effective than rhIL-3 in increasing the number of circulating hematopoietic progenitor cells BFU-E and CFU-GM. High-dose rhIL-3, but not low-dose rhIL-3, was as effective as the rhIL-3/rhGM-CSF combinations in increasing the number of circulating CFU-GEMM. The increase in absolute neutrophil counts correlated with the increase in the number of circulating CFU-GM. Side effects, mainly fever, headache, flushing, and sweating, were generally mild, but in two patients the occurrence of chills, rigor, and dyspnea after initiation of GM-CSF treatment necessitated dose reduction and discontinuation, respectively. These results indicate that sequential treatment with rhIL-3 and rhGM-CSF is as effective as single-factor treatment with rhIL-3 in stimulating platelet counts, whereas the effect of combination therapy on neutrophil counts and circulating progenitor cells is superior.

HEMATOPOIESIS is regulated by proteins that control cell proliferation and differentiation.[1] Although some of these factors have relatively restricted biologic activities, other factors such as interleukin-3 (IL-3)[2] and GM-CSF[3] have a broader range of action, stimulating multiple cell lineages. In addition to their direct effects,[4-9] in vitro[10-12] and preclinical in vivo[13-19] studies have also shown synergistic activity of these factors when applied simultaneously, either owing to direct synergism or to induction of further cytokines by accessory cells. Because IL-3 acts on an earlier progenitor cell population than does GM-CSF,[6,11,12] sequential exposure first to IL-3 and then to GM-CSF might have the advantage of expanding the GM-CSF–responsive progenitor and precursor cell pool, resulting in synergistic action of both cytokines.

In a previous phase I trial with recombinant human IL-3 (rhIL-3), stimulation of bone marrow (BM) progenitor cells and induction of neutrophil, platelet, and reticulocyte increases by rhIL-3,[20] but also lack of considerable increase of circulating hematopoietic progenitor cells,[21] could be demonstrated. GM-CSF was more restricted in its action, leading to an increase in the number of circulating neutrophils, eosinophils, and monocytes.[22] The time course and extent of the hematopoietic effects as well as results of preclinical sequential administration of both IL-3 and GM-CSF to primates led to the conclusion that IL-3 expands an early progenitor cell population which can then be stimulated by the later acting GM-CSF for further expansion.[14,15,18,19]

To analyze the effect of sequential administration of rhIL-3 and rhGM-CSF on the progenitor cell compartments and the mature cell populations, patients first received a course of rhIL-3 alone, followed by a sequential treatment with rhIL-3 and rhGM-CSF. The results of this trial indicate that sequential administration of both cytokines potentiates the stimulatory activity of IL-3 on circulating progenitor cells and neutrophils, with preservation of its capacity to stimulate thrombopoiesis.

MATERIALS AND METHODS

Patient selection. In a phase I study, 15 patients (11 men and four women, ranging in age from 21 to 76 years [median, 57 years 5 months]) with advanced malignancies and preserved hematopoietic function were treated with rhIL-3 followed by rhIL-3/rhGM-CSF. The underlying diseases and previous therapies are shown in Table 1. Eight of the 15 patients had received cytotoxic therapy for 5 to 88 weeks (median, 10.5 weeks) before initiation of cytokine treatment. The first course of rhIL-3 was not administered during the recovery phase from previous chemotherapy in any of these patients. The other seven patients had not received any myelosuppressive or immunomodulatory medication. None of the patients had clinical evidence of bacterial, fungal, or viral infections when entering this study. Apart from a slightly reduced mean hemoglobin concentration, there were no signs of hematopoietic dysfunction. None of the patients received cytotoxic treatment between the first and second cycle of cytokine therapy. One patient with preserved hematopoietic function treated at an rhIL-3 dose level of

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Table 1. Patient Characteristics

Patient	IL-3 ($\mu g/m^2$)	Age/Sex	Diagnosis	Chemotherapy*	Interval to IL-3 (wk)	Interval Between Cycles (wk)	GM-CSF Dose During Second Cycle ($\mu g/m^2/d$)
F40	60	73/F	Large cell cancer	—	—	5	2 × 250, 6 × 125
F41	60	51/F	Mesothelioma	—	—	4.5	10 × 250
F55	60	59/F	Adrenal cancer	—	—	3.5	10 × 125
F61	60	60/M	Gastric cancer	—	—	4.5	10 × 250
4/7	60	60/M	Liver cancer	FU	5	3	4 × 250
F65	125	53/M	Rectal cancer	FU, leukovorin	8	3	10 × 250
2/5	125	54/M	Pharyngeal cancer	Vindesine, mitomycin, platin, FU	7	3.5	10 × 250
4/2	125	21/M	Chordoma	—	—	2	10 × 250
4/4	125	57/M	Leimyosarcoma	Doxorubicin, ifosfamide	14	2	10 × 250
4/8	125	57/F	Histiocytoma	Doxorubicin	8	3	10 × 250
F8	250	66/M	NHL, adenocarcinoma	Chlorambucil, FU	12	3.5	10 × 250
F10	250	49/M	Renal cell cancer	Epirubicin, MTX	88	16	1 × 250
F16	250	76/M	Renal cell cancer	Vinblastine	20	7.5	2 × 250; 8 × 125
F31	250	57/M	Colon cancer	—	—	17	10 × 250
4/6	250	53/M	Adenocarcinoma	—	—	3	10 × 250

Abbreviations: FU, 5-fluorouracil; MTX, methotrexate.

250 $\mu g/m^2$ was evaluable for toxicity but not for hematologic response because rhGM-CSF had to be discontinued after 1 day of treatment with rhGM-CSF.

Eligibility criteria included a performance status of more than 50% (Karnofsky scale); life expectancy of more than 3 months; preserved hepatic, renal, cardiac, and hemostaseologic function; and absence of clinically apparent allergies or bronchoalveolar disorders. The study was approved by the Ethics Committee of the University of Frankfurt. Informed written consent was obtained from the patients before rhIL-3 therapy was started.

Recombinant human IL-3. The cDNA of rhIL-3 was isolated from human peripheral blood lymphocytes (PBL) and the gene product was expressed in yeast.[23,24] The molecular weight (mol wt) was in a range of 14 to 16 Kd, depending on the degree of glycosylation. The rhIL-3 used in this study was produced by Immunex (Seattle, WA) and provided by Behringwerke AG (Marburg, Germany). Specific activity of this material was $>1 \times 10^7$ U/mg protein in a bone marrow (BM) proliferation assay. Sterility, pyrogenicity, general safety, and purity studies met standard of the Office of Biologics. No endotoxin was measurable with the limulus amebocyte lysate assay (<10 pg/mg protein).

Recombinant human GM-CSF. The rhGM-CSF used was produced by Behringwerke AG. The recombinant protein was expressed in *Escherichia coli* and had a mol wt of 14 Kd.[25] Its specific activity was 5×10^7 U/mg protein, and it was free of detectable endotoxin.

Study design. Every patient received two cycles of cytokine treatment. rhIL-3 was administered by SC bolus injection daily for 15 days during the first cycle. The treatment schedule consisted of increasing dose levels of rhIL-3, ie, 60, 125, and 250 $\mu g/m^2$. During the second cycle, all patients received rhIL-3 at the same dosage as in the first cycle for 5 days, followed by treatment with rhGM-CSF at a daily dosage of 250 $\mu g/m^2$ for 10 consecutive days starting the day after the end of the rhIL-3 treatment.

Patients were monitored daily and all constitutional symptoms were recorded. Before and during the study, patients were regularly monitored by complete history and physical examination and laboratory tests, including a complete blood count, differential and reticulocyte counts, a chemistry profile, coagulation profile, and urinalysis. An electrocardiogram and chest roentgenogram were performed before the study and after the final dose.

Toxicity was graded according to the World Health Organization (WHO) criteria.[26] Dose-limiting toxicity was generally defined as toxicity of grade 3 or higher by WHO criteria. Antipyretics were not administered unless the body temperature increased to more than 39°C.

Progenitor cell assay. To determine the effect of cytokine treatment on the number of circulating hematopoietic progenitor cells (CFU-GEMM, BFU-E, and CFU-GM), low-density mononuclear cells were obtained from heparinized blood samples by a Ficoll-Hypaque density centrifugation (density 1.077 g/mL) before, after 7 days, and immediately after the end of the treatment cycles. In two patients, cell samples from the PB were obtained at more frequent time points. All cells were cryopreserved at a controlled freezing rate of 1°C/minute using a Cryoson programmable cryopreservation apparatus. The cultures were set up at the same time for all patients to prevent variations in culture conditions. Cells were cultured in a clonogenic methylcellulose assay system as described previously.[21] Mononuclear cells 2×10^5/mL were cultured in quadruplicate 1-mL aliquots in Iscove's modified Dulbecco's medium (IMDM) containing 1.1% methylcellulose, 30% fetal calf serum (FCS; Hyclone, Logan, UT), 50 μmol/L 2-mercaptoethanol, 10 ng/mL rhIL-3 (Behringwerke), 10 ng/mL rhGM-CSF (Behringwerke), 10 ng/mL rhG-CSF (Amgen, Thousand Oaks, CA), and 2 U/mL rh erythropoietin (Behringwerke). The culture plates were set up in quadruplicate. They were incubated for 14 days at 37°C and 5% CO_2 in a fully humidified atmosphere and scored in situ under an inverted microscope. Colonies derived from multipotent progenitors CFU-GEMM contained at least granulocytic/monocytic and erythroid elements; colonies derived from BFU-E contained more than 300 cells, whereas those derived from CFU-GM contained more than 50 cells.

Statistical analysis. Student's *t*-test and, where appropriate, the Wilcoxon signed-rank test for paired data were used to test for significant differences between data before and after administration of rhIL-3 and rhIL-3/rhGM-CSF, respectively. A least-squares linear regression analysis was performed to relate the relative increase in the number of neutrophilic granulocytes to the log-transformed increase in the number of circulating CFU-GM.

RESULTS

PBL counts and hemoglobin levels. The patients' clinical responses to therapy are shown in Tables 2 and 3 and Figs 1 and 2. In response to SC daily administration of rhIL-3 for 15 days, the leukocyte counts increased in a dose-

Table 2. Change in PB Counts and in Hemoglobin

IL-3 Dosage (μg/m²)/ Patient	Leukocytes (×10⁻³/μL)				Platelets (×10⁻³/μL)				Hemoglobin (g/dL)			
	IL-3 Cycle		IL-3/GM-CSF Cycle		IL-3 Cycle		IL-3/GM-CSF Cycle		IL-3 Cycle		IL-3/GM-CSF Cycle	
	Day 0	Day 15	Day 0	Day 15	Day 0	Day 15	Day 0	Day 15	Day 0	Day 15	Day 0	Day 15
60												
F40	9.5	16.8	10.5	66.9	348	499	424	447	11.6	12.5	12.7	12.7
F41	5.8	7.5	4.6	23.2	225	376	273	385	13.2	13.3	12.5	12.4
F55	9.7	10.9	10.0	32.7	369	458	362	378	13.2	11.7	12.0	14.4
F61	5.4	7.1	5.9	33.7	302	389	293	427	13.1	13.8	14.5	15.9
4/7	5.6	7.8	9.8	31.3	147	227	212	263	9.2	9.0	9.4	10.2
Mean ± SEM	7.2 ± 1.0	10.0‡ ± 1.8	8.2 ± 1.2	37.6‡§ ± 7.6	278 ± 41	390‡ ± 47	313 ± 37	380‡ ± 32	12.1 ± 0.8	12.1 ± 0.8	12.1 ± 0.8	13.0 ± 0.9
125												
F65	13.9	19.0	9.8	37.1	603	677	513	392	8.4	9.4	10.6	10.7
2/5	5.8	10.5	7.6	20.3	197	339	227	171	16.3	16.1	16.6	16.6
4/2	7.2	10.5	15.4	34.2	286	443	363	352	12.4	10.1	10.8	9.8
4/4	5.8	28.7	9.1	56.5	262	355	421	384	9.5	9.9	10.0	10.5
4/8	5.0	10.9	5.9	54.8	398	631	328	442	12.7	13.3	13.5	14.1
Mean ± SEM	7.5 ± 1.6	15.9‡ ± 3.6	9.6 ± 1.6	40.7‡§ ± 6.8	349 ± 71	489‡ ± 70	370 ± 48	348 ± 42	11.9 ± 1.4	11.8 ± 1.3	12.3 ± 1.2	12.3 ± 1.3
250												
F8	11.2	17.3	14.8	55.6	269	379	233	428	12.8	12.3	11.8	13.1
F10*	3.5	12.1	4.6	6.2	200	549	230	258	11.9	11.6	11.1	9.4
F16	6.0	23.1	4.9	37.1	247	556	238	507	13.6	12.7	14.8	13.5
F31	6.3	8.8	6.2	35.1	343	434	312	307	13.7	13.0	14.1	15.4
4/6	7.2	12.4	5.8	51.2	298	416	269	316	15.6	15.2	14.6	16.1
Mean ± SEM	7.7 ± 1.2	15.4‡ ± 3.1	7.9 ± 2.3	44.8‡§ ± 5.1	289 ± 21	446‡ ± 38	263 ± 18	390‡ ± 48	13.9 ± 0.6	13.3 ± 0.6	13.8 ± 0.7	14.5 ± 0.7

*Received only one dose of rhGM-CSF during the rhIL-3/rhGM-CSF treatment cycle.
†Excluding data from patient F10.
‡Significantly higher than on day 0 ($P < .05$).
§Significantly higher than after treatment with IL-3 alone ($P < .05$).

dependent manner (Table 2), primarily due to a dose-dependent increase in banded and segmented neutrophils and eosinophils (Table 3). Circulating neutrophil levels increased up to 2.2-fold at 125 μg/m² and 1.7-fold at 250 μg/m². Although band neutrophils increased, more immature myeloid cells, ie, myelocytes and metamyelocytes, were only rarely detectable in the blood smears. As shown in Fig 1, more than 1 week elapsed before the neutrophil counts increased conspicuously. After discontinuation of treatment, neutrophil numbers returned to baseline levels in 1 week. Reversible eosinophilia occurred at all dose levels, ranging from 1,200/μL to 3,800/μL. Basophil counts also increased but remained in the normal range.

During the second treatment cycle, during which a 5-day course of rhIL-3 was immediately followed by a 10-day course of rhGM-CSF, the PBL counts increased dramatically between 4.2-fold and 5.7-fold after the switch from rhIL-3 to rhGM-CSF treatment, reaching maximum values immediately after the end of rhGM-CSF treatment that were significantly higher than during the first cycle but not different between the three treatment groups (Table 2). In particular, there was a rapid increase in segmented neutrophils (Fig 2) and a concomitant shift to the left, with appearance of myelocytes, metamyelocytes, and band forms (Fig 1). Although all patients received the same dosage of rhGM-CSF, at the end of the 10-day GM-CSF treatment there was still a difference between the three treatment groups: The absolute number of neutrophilic granulocytes, both immature and mature, as well as the degree of the shift to the left were related to the dosage of rhIL-3 administered during the first 5 days (Table 3). The higher rhIL-3 dosages were associated with more pronounced responses of the neutrophilic granulocytes.

The degree of eosinophilia was dependent on the dosage of IL-3 in each treatment cycle and was augmented by the subsequent treatment with rhGM-CSF (Table 3). In this respect, the increase in eosinophils closely resembled the increase in neutrophils. The changes in monocyte counts were minor during both treatment cycles, but their increase was primarily dependent on treatment with GM-CSF. Basophil counts increased to the same extent during both cycles whether only IL-3 or both IL-3 and GM-CSF were

Table 3. Leukocyte Counts per Microliter (mean ± SEM) Before and After IL-3 of IL-3/GM-CSF Therapy

Cell	IL-3 (60)		IL-3/GM-CSF (60)/(250)		IL-3 (125)		IL-3/GM-CSF (125)/(250)		IL-3 (250)		IL-3/GM-CSF (250)/(250)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Neutrophils												
Segmented	4.9 ± 1.2	4.2 ± 0.8	5.0 ± 1.4	15.8 ± 2.8*†	5.9 ± 1.3	9.8 ± 2.5*	7.2 ± 1.2	21.6 ± 3.4*†	5.1 ± 1.3	7.8 ± 1.5*	5.1 ± 1.3	19.5 ± 3.2*†
Band forms	0	0.2 ± 0.1	0.1 ± 0.1	2.3 ± 0.3*†	0.1 ± 0.1	1.3 ± 0.5*	0.1 ± 0.1	4.1 ± 1.3*†	0.1 ± 0.1	0.9 ± 0.6*	0	7.1 ± 2.7*†
Myelocytes/ metamyelocytes	0	0	0	0.7 ± 0.4*†	0	0.1 ± 0.1	0	0.9 ± 0.8*	0	0.1 ± 0.1	0	1.3 ± 0.2*†
Eosinophils	0.2 ± 0.2	1.2 ± 0.3*	0.3 ± 0.1	5.0 ± 1.5*†	0.3 ± 0.1	2.0 ± 0.8*	2.1 ± 1.2	8.5 ± 4.0*†	0.1 ± 0.1	3.8 ± 1.7*	0.4 ± 0.2	9.4 ± 1.6*†
Basophils	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.4 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.5 ± 0.2*	0	0.7 ± 0.2*
Monocytes	0.5 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	1.6 ± 0.4*†	0.6 ± 0.2	0.9 ± 0.2	0.6 ± 0.1	1.2 ± 0.3*	0.7 ± 0.2	0.6 ± 0.2	0.4 ± 0.1	1.5 ± 0.1*†
Lymphocytes	1.5 ± 0.2	2.0 ± 0.5	1.4 ± 0.4	2.1 ± 0.5	1.1 ± 0.1	1.6 ± 0.3	1.0 ± 0.1	3.0 ± 0.7*†	2.4 ± 0.7	3.3 ± 0.8	2.1 ± 0.9	5.2 ± 1.8*†

Abbreviations: Pre, before; Post, after.
*Significantly increased above pretreatment value ($P < .05$).
†Significantly higher than after treatment with IL-3 alone ($P < .05$).

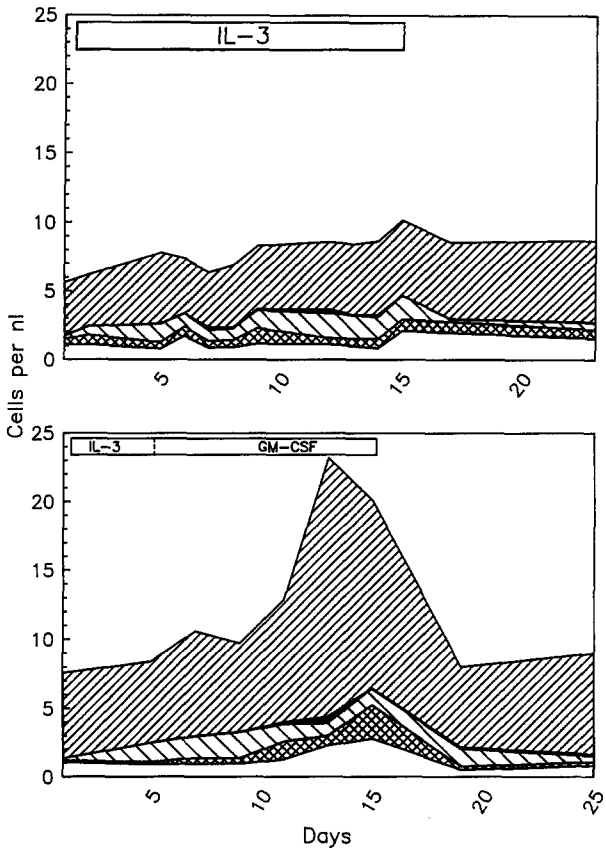

Fig 1. Change in total leukocyte count and differential in patient F 2/5 treated with either 60 μg rhIL-3/m²/d for 15 days (top), or with 60 μg rhIL-3/m²/d for 5 days followed by 250 μg rhGM-CSF/m²/d for 10 days (bottom). (▨) Segmented neutrophils; (■) basophils; (▧) eosinophils; (▩) monocytes; (□) lymphocytes.

administered. Although lymphocyte counts increased during both modes of cytokine treatment, the increases were more pronounced when IL-3 was followed by GM-CSF.

Whereas there was virtually no change in the mean hemoglobin levels after treatment with rhIL-3 alone, hemoglobin levels increased by more than 1 g/dL in five patients receiving the sequential treatment schedule. At the highest rhIL-3 dose level, three of four evaluable patients actually exhibited an increase in their hemoglobin concentration (Table 2).

Platelet counts. During the treatment cycle with rhIL-3 alone, a dose-dependent increase in maximum platelet counts was observed, ranging from 1.5-fold at 60 and 125 μg/m² to 1.8-fold at 250 μg/m² (Table 2). As shown in Fig 2, the time until an increase in platelet numbers became evident was equally dose-dependent, with earlier increases at the two higher dosages. The platelet counts continued to increase for an additional week after discontinuation of rhIL-3 treatment before returning to baseline levels over a period of 2 to 3 weeks.

An important aspect of the current study was a comparison of the platelet responses during treatment with rhIL-3 alone and the sequential rhIL-3/rhGM-CSF treatment. At the 60-μg/m² level of rhIL-3, the platelet response during both treatment cycles was virtually identical, with a maximum 1.5-fold increase in platelet counts and a prolonged return to baseline during both cycles (Fig 2). Similarly, at the 250-μg/m² dose level, the curves were superimposable up to day 15, with a 1.5-fold increase of platelet counts during the rhIL-3/rhGM-CSF cycle *v* a 1.6-fold increase during the IL-3 cycle.

In contrast, no change in platelet counts was observed during the IL-3/GM-CSF treatment course at the 125-μg/m² level of rhIL-3 (Table 2 and Fig 2). The main

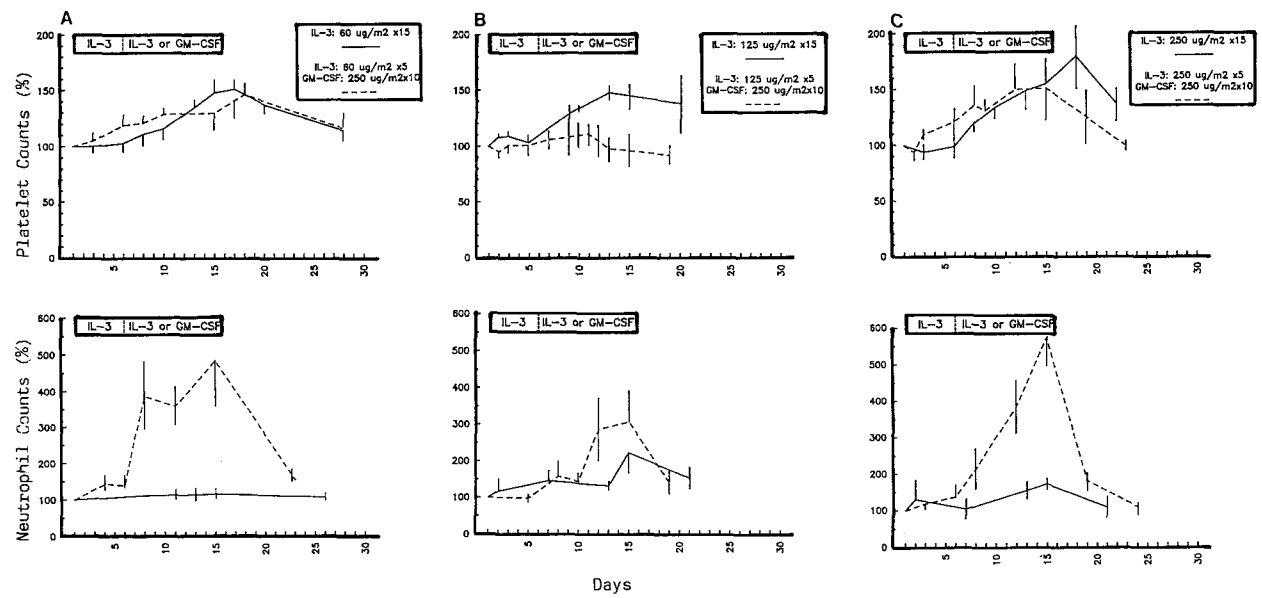

Fig 2. Effect of rhIL-3 and sequential combination therapy with rhIL-3 and rhGM-CSF on the platelet and absolute neutrophil counts treated at three different doses of rhIL-3: 60 μg/m² (A); 125 μg/m² (B); 250 μg/m² (C). The dosage of rhGM-CSF was 250 μg/m². Initial values were set at 100%. Tables 2 and 3 show absolute counts.

difference between the group treated at this level as compared with the other two dose levels was the significantly shorter time interval between the two treatment cycles, ranging from 2 to 3.5 weeks with a mean of 2.7 weeks versus 4.1 and 9.4 weeks in the other groups ($P < 0.05$). Apparently, this shorter time interval also explains the eosinophilia and basophilia evident at the beginning of the second treatment cycle in this group as compared with the two other groups (Table 3).

Circulating progenitor cells. The frequencies of circulating progenitor cell subsets were assessed in maximally stimulated clonogenic assays. Table 4 shows the results in eight patients with intact hematopoietic function, four treated at the 60-μg/m² rhIL-3 dose level, one at the 125-μg/m² rhIL-3 dose level, and three at the 250-μg/m² dose level. Because the hematologic responses observed in the latter four patients were identical, the progenitor cell changes of these patients were combined and compared with those at the lowest dose level. After rhIL-3 treatment at a dosage of 60 μg/m², the mean number of CFU-GEMM decreased to 93% ± 58% and 28% ± 11% on day 8 and 15, respectively. In contrast, during the second treatment course, CFU-GEMM increased to 321% ± 151% and 747% ± 477% of day 0 levels after 8 and 15 days. Similarly, at 60 μg/m², the day 8 and day 15 values for BFU-E were 137% ± 34% and 592% ± 360% during the second treatment cycle, whereas the corresponding numbers after rhIL-3 treatment alone were 101% ± 25% and 196% ± 155% of day 0 levels. Numbers of CFU-GM per volume of blood increased to 194% ± 39% and further to 2,765% ± 2,443% after 8 and 15 days of IL-3/GM-CSF therapy, whereas CFU-GM increased to 284% ± 140% and 307% ± 253% during IL-3 treatment alone.

Treatment at the higher dosages of rhIL-3 had a more pronounced effect on the number of circulating progenitor cells. After 8 and 15 days of treatment with rhIL-3 at a daily dosage of either 125 or 250 μg/m², the mean number of circulating CFU-GEMM increased to 175% and 817% ± 337%, whereas during the combined treatment cycle with rhIL-3 and rhGM-CSF these increases were comparable, reaching 252% ± 137% and 737% ± 475% of day 0 values. Although the number of circulating BFU-E were increased only moderately by this dose level of rhIL-3 (64% after 8 days and 196% ± 48% after 15 days), addition of rhGM-CSF led to a rapid increase in the number of circulating BFU-E after 8 days (368% ± 141%) and 15 days (609% ± 257%). The potentiating effect of rhGM-CSF became even more apparent when changes of circulating CFU-GM were examined: whereas rhIL-3 treatment alone increased the number of circulating CFU-GM to 206% and 457% ± 86%, the corresponding day 8 and day 15 values after the combined treatment course were 386% ± 141% and 2,916% ± 2,245%.

Comparison of the various sequences and rhIL-3 dose ranges showed that higher dose rhIL-3 administered alone was superior to low-dose rhIL-3 in increasing the number of circulating CFU-GEMM, but equally effective as the sequential rhIL-3/rhGM-CSF regimens (Fig 3). In contrast, sequential rhIL-3/rhGM-CSF was superior to the corresponding rhIL-3 courses in augmenting the number of circulating BFU-E and CFU-GM, whereas there was no difference between sequential courses using high-dose or low-dose rhIL-3. The relative increase in the number of circulating CFU-GM correlated significantly with the relative increase in the number of neutrophilic granulocytes ($r = .806$, $P < .01$) (Fig 4). Although hemoglobin concentrations increased in six of 11 treatment cycles in which circulating BFU-E increased, and during only one of five cycles in which the number of circulating BFU-E decreased, this difference did not reach the level of significance.

To elucidate the possible kinetics of the response of the circulating progenitor cell populations to treatment with rhIL-3 alone versus sequential therapy with rhIL-3 and rhGM-CSF, the number of progenitors per volume of blood was studied at more frequent time intervals in two individual patients, one treated at the 60-μg/m² rhIL-3 dose level and the other at the 125-μg/m² dose level (Fig 5). Although the changes in the number of circulating CFU-GEMM, BFU-E, and CFU-GM during and after treatment with rhIL-3 alone was in the expected range, the number of circulating progenitors was rapidly expanded by rhGM-CSF administration. The kinetics, however, differed between the two patients, with maximum values reached on day 8 in the patient receiving rhIL-3 at the dosage of 125 μg/m² whereas maximum numbers were not reached before day 15 in the patient at the 60-μg/m² rhIL-3 dose level.

Adverse effects of combination therapy. Toxicity of the treatment schedule was generally mild. Fever, not exceeding 40°C, was the most frequent adverse effect and was usually more pronounced during the first few days of

Table 4. Number of Circulating Progenitor Cells CFU-GEMM, BFU-E, and CFU-GM per Milliliter of PB

	CFU-GEMM						BFU-E						CFU-GM					
	IL-3			IL-3/GM-CSF			IL-3			IL-3/GM-CSF			IL-3			IL-3/GM-CSF		
Patient	Day 0	Day 8	Day 15	Day 0	Day 8	Day 15	Day 0	Day 8	Day 15	Day 0	Day 8	Day 15	Day 0	Day 8	Day 15	Day 0	Day 8	Day 15
F40	114	70	19	74	168	226	866	861	166	688	1,641	2,145	437	580	105	373	879	2,077
F41	31	63	6	9	19	3	508	494	110	251	278	124	180	339	56	41	112	163
F55	43	6	21	8	61	40	240	103	204	270	242	960	83	95	90	71	124	220
F61	0	0	0	16	13	344	32	52	211	130	142	2,157	11	78	117	15	14	1,469
F65	24	44	118	62	409	67	268	192	558	425	2,864	601	212	533	751	491	1,463	601
F8	13	ND	126	38	30	66	297	ND	590	477	314	1,053	220	ND	1,074	191	219	987
F16	18	30	22	20	21	427	318	176	223	305	1,615	3,682	40	64	119	20	156	1,920
F31	38	ND	640	19	31	101	710	ND	2,167	270	545	2,339	265	ND	1,819	80	281	1,141

Abbreviation: ND, not done.

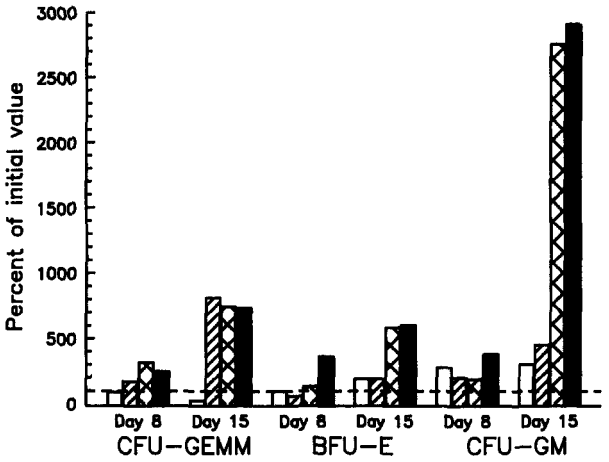

Fig 3. Effect of rhIL-3 and rhIL-3/rhGM-CSF treatment on the number of circulating progenitor cells after 8 and 15 days of treatment. Initial values were set at 100%. Table 4 shows absolute counts per milliliter of blood. (□) IL-3 60 μg/m² × 15; (▨) IL-3 125/250 μg/m² × 15; (▩) IL-3 60 μg/m² × 5, GM-CSF 250 μg/m² × 10; (■) IL-3 125/250 μg/m² × 5, GM-CSF 250 μg/m² × 10.

therapy (Table 5). Headache accompanied by neck stiffness was a common finding but did not necessitate a dose reduction. Several patients had mild local erythema at the site of SC injection of rhIL-3. In one patient treated at the 250-μg/m² dose level, development of chills, rigor, dyspnea, and fever after the first dose of rhGM-CSF necessitated discontinuation of therapy. The patient recovered completely in 24 hours but refused continuation of GM-CSF therapy at a lower dose. In two patients, the rhGM-CSF dosage was reduced to 125 μg/m² after the second dose owing to either chills and rigor or to dyspnea. Later, rhGM-CSF was discontinued in one of these patients after the eighth injection because of leukocytosis of 66,900/μL. For no apparent reason, one patient asked for discontinuation of treatment after the fourth dose of rhGM-CSF.

DISCUSSION

Because an important indication for using hematopoietic growth factors is prevention of prolonged cytopenia after high-dose chemotherapy without or with BM transplantation, it would be advantageous to increase the speed of recovery of all hematopoietic cell lineages maximally. GM-CSF and G-CSF mainly accelerate neutrophil recovery but lack a pronounced effect on thrombopoiesis.[27] The platelet response observed after treatment with rhIL-3 and the effect of IL-3 on the myeloid progenitor and precursor cells but not on late myeloid maturation have led us to the approach of combining IL-3 with GM-CSF sequentially to enhance a multilineage stimulatory effect. This approach was further supported by the in vitro analyses[10-12] and in vivo primate studies[14,15,18,19] which indicated that IL-3 and GM-CSF synergistically enhance hematopoietic stem cell proliferation and differentiation.

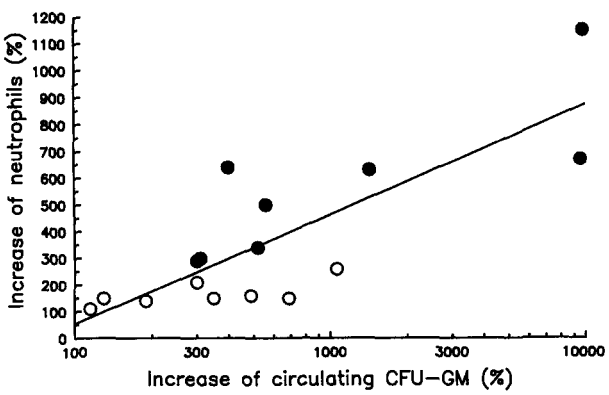

Fig 4. Correlation between the relative increases in the number of circulating CFU-GM and absolute neutrophil counts during treatment with IL-3 (○) or IL-3/GM-CSF (●) (r = .806, P < .01).

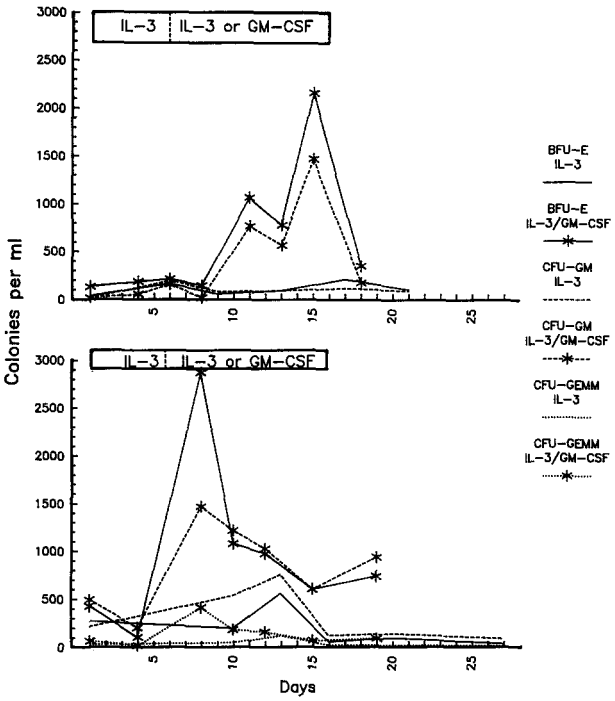

Fig 5. Effect of rhIL-3 and rhIL-3/rhGM-CSF treatment on the number of circulating progenitor cells in patient F61 treated at the 60-μg/m² dose level (A) and in patient F65 treated at the 125-μg/m² dose level (B).

As observed in our previous phase I trial, SC administration of rhIL-3 for 15 days at doses between 60 and 250 μg/m² induces a dose-dependent multilineage response with leukocyte as well as platelet responses.[20] The myelostimulatory effect of sequential IL-3/GM-CSF treatment was significantly higher than IL-3 therapy alone, but there was no substantial difference in maximum leukocyte counts between the three different IL-3 dosages when used in combination with GM-CSF. Only when the neutrophilic and eosinophilic granulocytes and the lymphocytes were examined were the differences in IL-3 dosages apparent. Because the maximum leukocyte counts obtained during the IL-3/GM-CSF treatment were identical to those obtained in our previous phase I trial in which we treated patients with 250 μg/m² GM-CSF for 10 days,[22] IL-3 most probably requires later acting cytokines for expansion of mature blood cells.

Table 5. Adverse Effects

	Cycle 1				Cycle 2							
	rhIL-3				rhIL-3				rhGM-CSF			
Symptom	I	II	III	IV	I	II	III	IV	I	II	III	IV
Fever	3	3	—	—	2	3	—	—	2	1	—	—
Headache	—	2	—	—	3	—	—	—	—	—	—	—
Local erythema	3	—	—	—	—	—	—	—	—	—	—	—
Flush	2	—	—	—	3	—	—	—	—	1	—	—
Lethargy	1	—	—	—	2	1	—	—	1	1	—	—
Sweating	1	—	—	—	—	—	—	—	3	—	—	—
Diarrhea	—	—	—	—	1	—	—	—	1	—	—	—
Bone pain	—	—	—	—	1	—	—	—	—	—	—	—
Itching	—	—	—	—	1	—	—	—	—	—	—	—
Nausea	—	—	—	—	1	—	—	—	—	—	—	—
Chills, rigor	—	—	—	—	1	—	—	—	—	—	2	—
Dyspnea	—	—	—	—	—	—	—	—	—	—	1	—
Gastric pain	—	—	—	—	—	—	—	—	—	1	—	—

Although reticulocyte counts increased in most patients, this translated into an increase of hemoglobin values only in a few patients. Nevertheless, five patients exhibited a substantial increase in hemoglobin concentrations of more than 1 g/dL during the sequential treatment course as compared with only one patient during single IL-3 treatment. Especially three of four patients receiving the highest rhIL-3 dose followed by rhGM-CSF responded with an increase in hemoglobin concentrations, whereas no such increase was observed during their initial treatment with rhIL-3 alone. Because these data are limited, further studies with either higher dosages of IL-3 or combinations with erythropoietin are needed.

The most important aspect of this trial was to determine whether a 5-day course of IL-3 followed by GM-CSF treatment was as or more effective in stimulating thrombopoiesis as compared with a 15-day course of IL-3 alone. At two IL-3 dose levels (ie, 60 and 250 $\mu g/m^2$), the sequential treatment course with shortened IL-3 administration was indeed as effective in stimulating thrombopoiesis, with no statistical difference in the platelet increases between the first and the second treatment cycle, implying that shortening the period of IL-3 treatment from 15 to 5 days was sufficient for stimulation of platelet formation. Our observation of increased IL-6 serum levels during IL-3 treatment[28] indicate that this thrombopoietic effect of IL-3/GM-CSF might be mediated by secondary cytokines.

The course of the platelet counts during the combined IL-3/GM-CSF cycle at the 125-$\mu g/m^2$ IL-3 dose level apparently contradicts some of these conclusions, since the platelet counts did not increase during the second treatment cycle. The treatment-free interval between the first and second treatment course was considerably shorter, however, in contrast to the other dose levels in which the minimum time period between the cycles was 3 weeks. As a result, the stimulation of hematopoiesis induced by the first IL-3 treatment course was probably still apparent, as demonstrated by the higher number of neutrophils, eosinophils, basophils, and platelets at the start of the second treatment cycle. Because the platelet response to IL-3 is partially dependent on the initial platelet counts (ie, higher initial platelet counts are associated with a more moderate platelet response to IL-3[20]), this prestimulation of thrombopoiesis is most probably responsible for the lack of any further stimulation of platelet counts during the second cycle. An additional conclusion may be that there is no advantage in prolonging IL-3 therapy for too long before switching to a later acting cytokine such as GM-CSF; thus, IL-3 treatment could be restricted to 5 days or possibly fewer. The minimum number of days has not yet been established, however.

With regard to the effect on circulating progenitor cells, the higher dose of IL-3 was superior to lower dose IL-3 and as efficient as the sequential IL-3/GM-CSF treatment in stimulating the pluripotent progenitor cells CFU-GEMM. This preferential increase of pluripotent progenitors is an indication of the target cell population of IL-3, which is restricted to the more immature cells in comparison to the target cell population of GM-CSF.[6,11,12]

In contrast to the behavior of the circulating CFU-GEMM, the increase in the number of circulating unipotent progenitor cells BFU-E and CFU-GM was more pronounced when IL-3 treatment was followed by GM-CSF. This was most obviously true of the number of circulating CFU-GM, which could be increased 28-fold to 29-fold. Although the effect of a treatment course with GM-CSF alone was not established in these patients, comparisons with published reports[29-31] and especially the study by Haas et al,[32] who used the same GM-CSF material at the identical dosage, indicate that the 28-fold to 29-fold increase in circulating CFU-GM is higher than would have been expected from treatment with GM-CSF alone. The increase in circulating BFU-E was higher than that reported by Villeval et al[31] but similar to the changes reported by Socinski et al[29] who both, however, used much higher dosages of GM-CSF in most of their patients.

The observed correlation between the increase in the number of circulating CFU-GM and absolute neutrophil counts indicates stimulation of the entire granulocytic lineage by sequential IL-3/GM-CSF therapy. In the case of single-factor therapy with IL-3, this stimulation might be restricted to the more immature granulocytic cells, whereas

the increase of mature neutrophils, lacking IL-3 receptors,[33] could be mediated through release of secondary cytokines. Despite the tendency of a correlation between the increase in the number of circulating BFU-E and the increase in hemoglobin concentration, the numbers are too small for a definite conclusion.

Although our data indicate that the stimulatory effect of sequential IL-3/GM-CSF therapy is reproducible, the two examples, in which the time course of the progenitor cell response to cytokine treatment was followed more closely, indicate that there can be differences between individuals in the time course of the progenitor cell response. Some patients might respond earlier than others, rendering it especially difficult to predict the best time for collecting cells such as circulating progenitor cells for peripheral stem cell transfusion. According to findings in patients treated with GM-CSF,[29,30] the response might be even more pronounced in patients who have undergone cytostatic therapy immediately before cytokine treatment.

Sequential therapy with rhIL-3 and rhGM-CSF was generally well tolerated; however, the well-known side effects of GM-CSF[27] appear to be slightly aggravated, most likely owing to prestimulation of the monocyte/macrophage system by IL-3. Although most patients received 250 $\mu g/m^2$ rhGM-CSF, in several patients this dosage was reduced to 125 $\mu g/m^2$ with no apparent decrease in the granulocyte response. Therefore, this lower dosage of GM-CSF may suffice for a maximum response in a better tolerated dose range. On the other hand, after cytoreductive therapy, these adverse effects are probably much less pronounced owing to the absence of a responsive effector cell population, similar to the findings when GM-CSF is used after chemotherapy.[27]

These are the first data obtained in patients after treatment with an early and a later acting hematopoietic growth factor. The schedule of sequential application was chosen based on data obtained in primates.[14,15] Although competition between IL-3 and GM-CSF for receptor binding has been described,[34] this generally does not appear to impair in vitro and in vivo response of hematopoietic progenitor and precursor cells. Simultaneous administration of IL-3 and GM-CSF or administration of fusion proteins such as PIXY-321[35] may even accelerate leukocyte response further while simultaneously stimulating thrombopoiesis. Cytokines which might be effectively combined with IL-3 include erythropoietin to stimulate erythropoiesis[36] and IL-6 to stimulate thrombopoiesis,[37] M-CSF[38] and G-CSF. Future carefully designed clinical trials will have to clarify the potential of combination therapy of hematopoietic growth factors.

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Sequential in vivo treatment with two recombinant human hematopoietic growth factors (interleukin-3 and granulocyte-macrophage colony- stimulating factor) as a new therapeutic modality to stimulate hematopoiesis: results of a phase I study

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