

A Randomized Controlled Phase III Trial of Recombinant Human Granulocyte Colony-Stimulating Factor (Filgrastim) for Treatment of Severe Chronic Neutropenia

By David C. Dale, Mary Ann Bonilla, Mark W. Davis, Arline M. Nakanishi, William P. Hammond, Joanne Kurtzberg, Winfred Wang, Ann Jakubowski, Elliott Winton, Parviz Lalezari, William Robinson, John A. Glaspy, Steve Emerson, Janice Gabrilove, Martha Vincent, and Laurence A. Boxer

Patients with idiopathic, cyclic, and congenital neutropenia have recurrent severe bacterial infections. One hundred twenty-three patients with recurrent infections and severe chronic neutropenia (absolute neutrophil count $< 0.5 \times 10^9/L$) due to these diseases were enrolled in this multicenter phase III trial. They were randomized to either immediately beginning recombinant human granulocyte colony-stimulating factor (filgrastim) (3.45 to 11.50 $\mu\text{g}/\text{kg}/\text{d}$, subcutaneously) or entering a 4-month observation period followed by filgrastim administration. Blood neutrophil counts, bone marrow (BM) cell histology, and incidence and duration of infection-related events were monitored. Of the 123 patients enrolled, 120 received filgrastim. On therapy, 108 patients had a median absolute neutrophil count of \geq

$1.5 \times 10^9/L$. Examination of BM aspirates showed increased proportions of maturing neutrophils. Infection-related events were significantly decreased ($P < .05$) with approximately 50% reduction in the incidence and duration of infection-related events and almost 70% reduction in duration of antibiotic use. Asymptomatic splenic enlargement occurred frequently; adverse events frequently reported were bone pain, headache, and rash, which were generally mild and easily manageable. These data indicate that treatment of patients with severe chronic neutropenia with filgrastim results in a stimulation of BM production and maturation of neutrophils, an increase in circulating neutrophils, and a reduction in infection-related events.

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SEVERE CHRONIC neutropenia includes a heterogeneous group of hematologic diseases characterized by a selective decrease in circulating neutrophils to levels often associated with recurrent fevers, chronic oropharyngeal inflammation, and severe infections.^{1,2} Severe chronic neutropenia has been divided into three main syndromes: idiopathic neutropenia,^{3,4} cyclic neutropenia,⁵⁻⁷ and congenital forms of neutropenia.⁸⁻¹² Diagnosis of these separate entities requires a careful history to document duration of symptoms, serial measurement of absolute neutrophil counts (ANCs), and exclusion of other hematologic disorders, such as drug-induced agranulocytosis, myelodysplasia, myeloid and other malignancies, and autoimmune disorders. In general, the severity of symptoms and risk of serious infections are inversely proportional to the ANC, with the greatest problems recurring in patients with counts of less than $0.5 \times 10^9/L$. There is currently no predictably effective treatment for severe chronic neutropenia; bone marrow (BM) transplantation, with its attendant risks and paucity of suitable donors, has been used successfully for only a few patients with congenital neutropenia.^{13,14} Medical management of the neutropenias is mainly

symptomatic and consists of aggressive antibiotic treatment of febrile patients suspected of having bacterial infections. Other therapies of uncertain efficacy include glucocorticoids, lithium, androgenic steroids, immunoglobulins, and plasmapheresis.¹⁵⁻²¹

Granulocyte colony-stimulating factor (G-CSF) is a hematopoietic growth factor with the capacity to promote the growth and maturation of myeloid cells and, in particular, the proliferation and differentiation of neutrophil progenitors both in vitro and in vivo. Human G-CSF has been purified,²² molecularly cloned,²³ and successfully expressed in *Escherichia coli*²⁴ allowing for production of large quantities of homogeneous purified recombinant human G-CSF (rhG-CSF [filgrastim]; Amgen, Thousand Oaks, CA). Highly purified native human G-CSF and rhG-CSF have similar in vitro biologic activity.²⁴

Phase I/II clinical trials showed that filgrastim appeared to be effective for treatment of patients with diagnoses of idiopathic,²⁵ cyclic,²⁶ and congenital²⁷ forms of neutropenia. Therefore, a randomized phase III clinical trial of the efficacy and safety of filgrastim in patients with severe chronic neutropenia was conducted. The primary objective of this study was to evaluate the efficacy of filgrastim to increase the median ANC to $\geq 1.5 \times 10^9/L$ in patients with severe chronic neutropenia. The secondary objectives were to evaluate the effects of this therapy on the prevention/resolution of secondary outcome variables, such as infections, hospitalizations, and antibiotic use, and to evaluate safety and tolerance of long-term, daily, subcutaneous injections of filgrastim.

MATERIALS AND METHODS

Patients. Adult and pediatric patients with a confirmed diagnosis of severe idiopathic neutropenia, cyclic neutropenia, or congenital neutropenia were eligible to participate in this trial. Specific diagnoses of the congenital forms of neutropenia included congenital agranulocytosis (Kostmann's syndrome),⁸⁻¹² Shwachman-Diamond syndrome,⁹ and myelokathexis.^{10,11} Entry criteria for all patients required documentation of severe neutropenia, defined as an ANC of less than $0.5 \times 10^9/L$ on three separate occasions over the previous 6 months. Patients with cyclic neutropenia were required to demon-

From the Departments of Medicine and Pediatrics, University of Washington, Seattle, WA; Memorial Sloan-Kettering Cancer Center, New York, NY; Duke University, Durham, NC; St. Jude's Children's Research Hospital, Memphis, TN; Emory University, Atlanta, GA; Montefiore Medical Center, New York, NY; University of Colorado, Denver, CO; University of California, Los Angeles, CA; University of Michigan, Ann Arbor, MI; and Amgen, Thousand Oaks, CA.

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Address reprint requests to David C. Dale, MD, Professor of Medicine, Department of Medicine RG-22, 1959 NE Pacific, University of Washington, Seattle, WA 98195.

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strate five consecutive days per cycle of ANC less than $0.5 \times 10^9/L$ for each of three regularly spaced cycles over a 6-month period. Additionally, patients were required to be symptomatic with at least three clinically significant infections treated with antibiotics or one life-threatening infection treated with intravenous antibiotics during the previous 12 months.

Patients were excluded from enrollment when there was evidence of chromosomal abnormalities, myelodysplasia, hematologic malignancy, aplastic anemia, systemic lupus erythematosus, or rheumatoid arthritis (Felty's syndrome) or if the neutropenia was drug induced. Patients with increased large granular lymphocytes (LGL) were included and categorized as idiopathic neutropenia if the lymphocyte count was less than $5.0 \times 10^9/L$. Patients with positive tests for antineutrophil antibodies were not excluded if they met other study criteria. The use of glucocorticoids, gamma globulin, or lithium within 1 month of enrollment and the use of other investigational drugs were proscribed. Normal renal function, hepatic function, blood coagulation, and cardiac function were required.

This study was approved by the Institutional Review Board of each participating center. Informed consent was obtained from each patient or parent/legal guardian when the patient was a minor.

Study design. If all eligibility criteria were met, patients were randomly assigned to one of two treatment groups. Patients randomized to group A began filgrastim therapy with a 1-month dosage equilibration period to determine the optimal dosage required to achieve an ANC $\geq 1.5 \times 10^9/L$, followed by a 4-month treatment period. Patients in group B began a 4-month observation period and received no filgrastim treatment for that period. After completion of the observation period, these patients received filgrastim therapy as defined for group A patients, beginning with the 1-month dosage equilibration period, followed by the 4-month treatment period. This treatment group is subsequently referred to as group B-X.

The starting dosages of filgrastim used for group A and group B-X patients were based on results from the phase I/II clinical trials²⁵⁻²⁷ and were idiopathic neutropenia, 3.45 $\mu\text{g}/\text{kg}/\text{d}$; cyclic neutropenia, 5.75 $\mu\text{g}/\text{kg}/\text{d}$; and congenital neutropenia, 11.50 $\mu\text{g}/\text{kg}/\text{d}$ administered twice a day. The dosage of filgrastim was adjusted to a level that maintained a median monthly ANC between 1.5 and $10.0 \times 10^9/L$. If the median ANC remained less than $1.5 \times 10^9/L$ for 2 weeks, the dosage was increased. If the median ANC achieved $10 \times 10^9/L$ or more for 4 weeks, the filgrastim dosage was decreased. All injections were administered by the subcutaneous route and the administered volumes were calculated from the patient's actual body weight on entry into the study. If the weight changed by more than 10% from this baseline, the administered volume was recalculated.

Prestudy assessments. Prestudy evaluations included a history, physical examination, and a panel of routine blood chemistry tests. A complete blood count including a manual differential, platelets, and reticulocytes was performed. A bone marrow (BM) aspirate was required to confirm the diagnosis and to assess hematopoietic cell morphology and myeloid to erythroid cell ratio. Baseline spleen size was determined by physical examination and/or by abdominal magnetic resonance imaging (MRI) or computed tomography (CT).

Patient monitoring. During the study, complete blood counts were obtained three times weekly during the 1-month dosage equilibration period and twice weekly during the 4-month observation period (group B) and the 4-month filgrastim treatment period (group A and group B-X). BM aspirates were analyzed at the end of the filgrastim treatment period. Blood samples for determination of antibodies to G-CSF were drawn before therapy, 1 month after the effective filgrastim dosage was achieved, and again at the end of the 4-month filgrastim treatment period. Testing for antibodies used a sensitive ¹²⁵I-rhG-CSF radioimmunoassay. Follow-up physical examinations and abdominal scans by MRI or CT to assess spleen size were made

at the conclusion of the observation period (group B) and again after the 4-month filgrastim treatment period (groups A and B-X).

Each patient maintained a diary, with daily recording of filgrastim dosing, predosing body temperature, concomitant medications, and adverse events. On an ongoing basis, the investigators reviewed and documented occurrence and duration of infections, antibiotic use, hospitalization, and clinically significant adverse events for each patient.

Adverse event rates are expressed as exposure-adjusted event rates per patient month (defined as a 28-day period). For a specific adverse event, exposure-adjusted event rates are calculated as the total number of events divided by the total study exposure in patient months.

Statistical analysis. The primary response criteria were prospectively defined and were based on the median ANC achieved over the 5-month filgrastim total treatment period (dosage equilibration plus treatment periods). A complete response was defined as a median ANC that was $\geq 1.5 \times 10^9/L$ and a partial response as a median ANC that was less than $1.5 \times 10^9/L$ but $\geq 0.5 \times 10^9/L$ and a minimum of 100% increase of the ANC over baseline. When the above criteria were not met, the patient was categorized as having no response. The secondary endpoints of this study were to demonstrate reductions in infection-related events and increases in BM parameters. Analyses were also performed for each diagnosis group. Two group B patients who did not receive filgrastim following the observation period were included in the between group comparisons but were not included in the within group comparisons. All analyses were performed using two-sided tests at a significance level of .05.

For the analyses of the median ANC and the incidence and duration of infection-related events, comparisons were made between the untreated group B and each of the two treated groups A and B-X. Data for the analysis of median ANC are presented as the medians of weekly medians for each patient over the 4-month observation period (group B) or over the 5-month total treatment period (groups A and B-X). Between group comparisons of groups A versus B used the Mann-Whitney U test. Within group comparisons of groups B versus B-X used the Wilcoxon signed-rank test.

For the analyses of the BM parameters, pretreatment values obtained before rhG-CSF treatment were compared with posttreatment values obtained within 1 month of the end of the 5-month filgrastim total treatment period for all patients (groups A and B-X combined) using the Wilcoxon signed-rank test.

The subgroup of congenital patients diagnosed with Kostmann's syndrome (also referred to as congenital agranulocytosis or severe congenital neutropenia) were separately analyzed. These patients were diagnosed on the basis of very severe neutropenia, marrow aspirates showing predominance of promyelocytes with little maturation to myelocytes and more mature neutrophilic cells (ie, early "maturation arrest") and often increased marrow and blood eosinophils.

RESULTS

Patient population. Between October 1988 and September 1990, 123 patients were entered into this study and were randomized either to group A, immediately starting filgrastim treatment, or to group B, delaying filgrastim treatment until after 4 months of observation. Of these, 120 patients received treatment with filgrastim. Three patients were withdrawn before receiving filgrastim treatment. One patient in group A was withdrawn because of not meeting the study entry criteria, one in group B because of protocol violations, and another in group B because of the reversal of neutropenia. Data collected during the observation period for the two group B patients are included in the analyses comparing groups A and B.

Demographic data on the 123 patients are listed in Table 1. The overall median age was 12.1 years; younger ages were noted in both the cyclic neutropenia and congenital neutropenia groups. Of the 123 patients, 54% were females. There was a female predominance among those diagnosed with idiopathic neutropenia, as noted previously,⁴ and there were more male patients with congenital forms of neutropenia. Thirty-six of the 60 patients with congenital forms of neutropenia were diagnosed with congenital agranulocytosis, or Kostmann's syndrome. The number of patients in the other subgroups of congenital neutropenia is shown in Table 1.

Neutrophil responses. The primary objective of this study was to determine the ability of filgrastim to increase these patients' median ANC to $\geq 1.5 \times 10^9/L$. In general, responses were apparent within a few days of beginning treatment. Table 2 shows the ANCs by diagnosis and treatment group for patients receiving filgrastim. Filgrastim treatment resulted in a greater than 16-fold increase in ANCs for all diagnoses in both groups A and B-X. The comparisons between treated and nontreated groups were statistically significant by both intragroup (groups B v B-X, $P \leq .001$) and intergroup (groups A v B, $P \leq .001$) comparisons.

Of the 120 treated patients, 108 showed a complete response (90.0%). Four additional patients with diagnoses of congenital neutropenia, two from group A and two from group B, demonstrated a partial response (3.3%). Only eight patients, one with a diagnosis of idiopathic neutropenia from group A, one with a diagnosis of cyclic neutropenia from group B, and six with a diagnosis of congenital neutropenia, five from group A and one from group B, failed to meet these response criteria (6.7%). Four of the six patients with congenital neutropenia were diagnosed with Kostmann's syndrome, one was diagnosed as having Shwachmann-Diamond syndrome, and one was diagnosed with possible autoimmune neutropenia subsequent to treatment initiation.

The median daily doses at the time of initial response to treatment for patients with idiopathic neutropenia was 3.45

Table 1. Demographic Information

Group/Diagnosis	N	Median Age (yr) (range)	No. of Females	No. of Males
All patients	123	12.1 (0.6-75.7)	67	56
<18 yr	75	7.2 (0.6-17.9)	34	41
<6 yr	31	2.2 (0.6-5.6)	14	17
A	63	11.3 (1.1-69.1)	34	29
Idiopathic	22	28.5 (2.1-69.1)	16	6
Cyclic	10	10.7 (2.5-47.2)	5	5
Congenital*	31	7.7 (1.1-32.9)	13	18
Kostmann's syndrome	20	7.6 (1.1-19.1)	7	13
B	60	14.0 (0.6-75.7)	33	27
Idiopathic	20	30.8 (1.3-75.7)	12	8
Cyclic	11	27.4 (10.5-59.1)	8	3
Congenital*	29	8.0 (0.6-33.8)	13	16
Kostmann's syndrome	16	5.3 (0.6-33.8)	6	10

* Congenital neutropenia includes patients with Shwachmann-Diamond syndrome (3), myelokathexis (4), glycogen storage disease type Ib (1), possible autoimmune neutropenia (1), and neutropenia with dysgammaglobulinemia (1).

Table 2. Absolute Neutrophil Counts

Group/Diagnosis	N	Median ANC ($\times 10^9$ cells/L) (min-max)
A	62	6.10 (0.03-19.44)*
Idiopathic	21	9.72 (0.03-16.32)*
Cyclic	10	9.88 (4.44-12.62)*
Congenital	31	3.45 (0.05-19.44)*
Kostmann's syndrome	20	3.08 (0.23-10.92)*
B	60	0.21 (0.00-1.55)
Idiopathic	20	0.23 (0.05-0.86)
Cyclic	11	0.67 (0.36-1.55)
Congenital	29	0.15 (0.00-0.98)
Kostmann's syndrome	16	0.07 (0.00-0.34)
B-X	58	8.67 (0.36-30.88)*
Idiopathic	19	11.48 (2.16-30.88)*
Cyclic	11	9.55 (0.65-16.10)*
Congenital	28	5.68 (0.36-16.10)*
Kostmann's syndrome	16	3.74 (0.36-16.10)*

For groups A and B-X results were obtained from the 5-month treatment period; group B values were during the 4-month observation period. Groups A and B were compared using a Mann-Whitney U test and groups B and B-X were compared used a Wilcoxon signed-rank test.

* $P \leq .001$.

$\mu\text{g/kg/d}$, cyclic neutropenia was 5.75 $\mu\text{g/kg/d}$, and congenital neutropenia was 11.50 $\mu\text{g/kg/d}$. The median dose at response for the Kostmann's group was also 11.50 $\mu\text{g/kg/d}$.

Changes in BM morphology. BM aspirates were evaluated for differential counts, neutrophil maturation, and myeloid to erythroid ratios (Table 3). The 88 patients who had both prefilgrastim and postfilgrastim treatment BM aspirates and differential counts completed were included in these analyses. The segmented neutrophils (polymorphonuclear neutrophils, PMNs), expressed as a percent of the BM differential count increased significantly after filgrastim treatment in all patients ($P \leq .001$). Within group differences were statistically significant for each of the three diagnosis groups ($P \leq .05$).

One index of the degree of maturation of the neutrophil series is the postmitotic to mitotic ratio, which is computed from the BM differential count as (metamyelocytes plus band forms plus segmented neutrophils)/(myeloblasts plus promyelocytes plus myelocytes). As shown in Table 3, overall there was a statistically significant increase in the postmitotic or maturation-storage compartment of the BM compared with the mitotic compartment ($P \leq .05$).

The median prestudy myeloid to erythroid ratios for the whole group of patients was 1.04 to 1, well below normal values (normal > 3 to 1^{28}). After treatment with filgrastim, the overall median myeloid to erythroid ratios were increased to normal (3.04 to 1, $P \leq .001$).

For the subgroup with Kostmann's syndrome, BM evaluations showed, overall, that maturation was less pronounced than with the other groups. However, the changes from baseline were statistically significant (Table 3).

Effects of filgrastim on infection-related morbidity. A secondary objective of this study was to determine the effects of treatment on the occurrence and duration of infections

Table 3. BM Aspirates

Diagnosis	N	% PMNs (median)		Postmitotic to Mitotic Ratio (median)			Myeloid to Erythroid Ratio (median)		
		Pre	Post	N	Pre	Post	N	Pre	Post
All patients	77	1.00	9.50*	82	0.81	1.61†	88	1.04	3.04*
Idiopathic	23	2.00	14.00†	25	1.11	2.32†	24	2.31	4.70†
Cyclic	14	3.50	14.00†	14	1.00	2.06†	14	1.18	2.90†
Congenital	40	0.00	5.25*	43	0.33	0.80	50	0.86	2.31†
Kostmann's syndrome	23	0.00	3.00*	24	0.06	0.32†	28	0.83	1.88†

Statistical analysis, using the Wilcoxon signed-rank test, compared before (Pre) and after (Post) filgrastim treatment.

* $P \leq .001$.

† $P \leq .05$.

and infection-related morbidity. Information on infection-related morbidity was obtained from daily diaries maintained by each patient and the medical records of the patients' physicians and hospitals to which they were admitted. The incidence and duration of infection-related events (eg, fever, abscess, bronchitis, conjunctivitis, gingivitis, otitis, pharyngitis, pneumonia, pyelonephritis, septicemia, sinusitis), antibiotic use (oral and intravenous), and hospitalizations were recorded.

The incidence and duration of infection-related events were significantly decreased with 5 months of filgrastim treatment (Table 4, A v B and B v B-X comparisons, $P \leq .05$). Filgrastim treatment also resulted in a statistically significant reduction in the incidence and duration of antibiotic use ($P \leq .001$). There were also reductions in infection-related events and antibiotic use for each diagnosis group, although only some of these differences were statistically significant (Table 4).

The median incidence and median duration of hospitalization were low. There was a statistically significant decrease in incidence of hospitalization for groups A and B-X (Table 4, $P \leq .05$). For patients with hospitalizations at any time during the observation or treatment periods, there was a statistically significant decrease in incidence of hospitalization for groups A and B-X ($P \leq .001$).

Safety analysis. Safety data were analyzed from the 120 patients who were treated with filgrastim. All patients reported adverse events during treatment; most of these events were not regarded as related to filgrastim. Events related to the treatment were generally mild and consisted of headache, general musculoskeletal pain, transient bone pain, and rash. The percentage of patients with these symptoms at some time during the 5-month treatment period was 41%, 38%, 33%, and 27%, respectively. The exposure-adjusted event rates (the total number of events divided by the total study exposure

Table 4. Infection-Related Events, Antibiotic Use, and Hospitalizations

Group/Diagnosis	N	Infection-Related Events		Antibiotic Use (oral + IV + IM)		Hospitalizations			
		Incidence (median no.)	Duration (median days)	Incidence (median no.)	Duration (median days)	Incidence		Duration	
						Mean No.	Median No.	Mean Days	Median Days
A	62	0.20*	3.40†	0.20*	3.40*	0.10	0.00†	1.09	0.00
Idiopathic	21	0.20†	1.40	0.20†	0.20†	0.08	0.00	0.50	0.00
Cyclic	10	0.30	4.20	0.40	3.40	0.06	0.00†	1.22	0.00
Congenital	31	0.20†	3.40	0.40†	5.00*	0.12	0.00	1.45	0.00
Kostmann's syndrome	20	0.40†	5.50	0.50	6.30†	0.13	0.00	1.58	0.40
B	60	0.50	6.63	0.49	9.83	0.24	0.00	1.72	0.00
Idiopathic	20	0.47	5.13	0.47	4.93	0.21	0.00	1.52	0.00
Cyclic	11	0.54	6.19	0.46	4.50	0.25	0.23	1.18	0.75
Congenital	29	0.70	7.64	0.67	13.30	0.25	0.00	2.05	0.00
Kostmann's syndrome	16	0.82	7.47	0.60	20.00	0.22	0.12	2.58	0.23
B-X	58	0.33†	2.86†	0.20*	3.19*	0.08	0.00*	1.58	0.00
Idiopathic	19	0.20†	1.00	0.20†	2.40	0.09	0.00	2.25	0.00
Cyclic	11	0.20	1.80	0.20	1.40	0.10	0.00†	1.23	0.00
Congenital	28	0.40†	5.20†	0.40	5.00*	0.06	0.00†	1.27	0.00†
Kostmann's syndrome	16	0.60	5.90	0.40	6.90†	0.06	0.00	1.35	0.00

For groups A and B-X results are obtained from the 5-month treatment period; group B values are during the 4-month observation period, and all are expressed on a per month basis. Groups A and B were compared using a Mann-Whitney U test and groups B and B-X were compared using a Wilcoxon signed-rank test.

* $P \leq .001$.

† $P \leq .05$.

in patient months) were, respectively, 35%, 25%, 17%, and 10% compared with 24%, 10%, 6%, and 4% for untreated patients.

On the basis of phase I/II studies that showed that chronic administration of filgrastim increased spleen size, the occurrence of splenomegaly was monitored carefully in these patients by physical examinations and/or MRI or CT. Before therapy, 18 patients had an enlarged spleen by palpation ranging from 1 to 4 cm below the left costal margin (BLCM). With therapy, palpable splenomegaly was reported in 29 patients, ranging from 1 to 5 cm BLCM. In five of nine centers, splenic volume was assessed by CT or MRI. The median percent increase in splenic volume among 59 patients so evaluated was 34%, ranging from 2% to 148%. The increase in the spleen size was generally asymptomatic; there was no evidence of infarction, hemorrhage, or predictable effects of the splenic enlargement on other blood cell counts during the 5-month treatment period.

Excluding patients with a single platelet count below $100 \times 10^9/L$, thrombocytopenia (≥ 2 counts less than $100 \times 10^9/L$) was noted in seven patients. Four had documented pre-study platelet counts less than $100 \times 10^9/L$. Two cases were mild ($>50 \times 10^9/L$), two were moderate (25 to $50 \times 10^9/L$), and three more were severe ($< 25 \times 10^9/L$), two of whom had Shwachman-Diamond syndrome and histories of thrombocytopenia prior to therapy. Although these patients required discontinuation of filgrastim for a few days, all subsequently returned to daily filgrastim therapy.

Serum samples from 113 of the 120 patients were tested for rhG-CSF antibody reactivity. Samples were collected before and after initiation of filgrastim treatment, ranging from 4 weeks to 9 months after the initial dosing period. None of the samples demonstrated rhG-CSF-specific reactivity.

DISCUSSION

This study demonstrates that, in contrast to any previous treatments, filgrastim is an effective and safe therapy for increasing blood neutrophils of patients with congenital, cyclic, and idiopathic neutropenia. In 108 of the 120 treated patients (90%), the median ANC was increased above the minimum study criteria ($1.5 \times 10^9/L$). Four additional patients had a partial response (median ANC $< 1.5 \times 10^9/L$ but $\geq 0.5 \times 10^9/L$ and a 100% increase from the baseline ANC). Only eight of the treated patients failed to meet the above response criteria. One nonresponding patient in the idiopathic group had large granular lymphocytosis, which has been reported to be associated with chronic idiopathic and acquired cyclic neutropenia.^{29,30} Six other patients who did not meet the criteria for even a partial response had congenital neutropenia. Only one patient with cyclic neutropenia failed to respond. However, most of these patients were regarded by the investigators to have less infection-related morbidity on therapy. Overall, these results closely parallel the finding of a complete response for 40 of 41 patients with these diagnoses treated in one institution and mentioned in a previous report.³¹

Analysis of the BM differential cell counts showed that filgrastim caused an increased percent of PMNs and an increased size of the postmitotic neutrophil pool, as reported previously for three patients with Kostmann's syndrome.³¹

These findings strongly suggest that the increased ANCs were due to increased neutrophil production. A direct action of filgrastim on the recruitment and maturation of neutrophil progenitor cells is the most likely explanation for these results, as suggested by previous *in vitro* and *in vivo* studies.^{23,24}

This study also showed that this large group of patients with severe chronic neutropenia and patients with each of the diagnoses studied benefited clinically from filgrastim therapy. The patients with idiopathic neutropenia demonstrated generally the lowest incidence and duration of antibiotic use after filgrastim treatment. These patients had the highest indices of BM maturation before treatment and responded to filgrastim with the greatest increase in ANCs. Also, the patients with idiopathic neutropenia required the lowest dosages of filgrastim to effect a complete response. On the other hand, the patients with congenital neutropenia who generally have the lowest ANCs and most severe problems with infections received the highest doses of filgrastim and had the highest duration of antibiotic use both before and after filgrastim treatment. These patients show significant reductions in infection-related events and antibiotic treatment. In cyclic neutropenia, median ANCs were increased and hospitalizations nearly eliminated. Other data for this group did not show statistically significant changes, but this was the smallest group and the trends in the data were all in the same direction as for the other patients.

Observations in this trial corroborate and extend data from previous phase I/II trials.^{25-27,32-42} Consistently across these phase I/II studies, most patients have responded to filgrastim but the degree of benefit, especially the clinical benefit, was unclear without a controlled clinical trial. Although the causes of these uncommon conditions are not known, the results of this phase III trial suggest a hierarchy of treatment responsiveness, which is reflected in the median doses required to achieve complete responses. In general, these differences correlate with the severity of the marrow defect as reflected by the marrow neutrophils, postmitotic to mitotic ratios, and myeloid to erythroid ratios for each group (Table 3). They suggest that there may be some minimal level of hematopoietic differentiation, eg, some minimal number and function of early neutrophil precursors, required for a response to filgrastim.

Neutrophil functional tests were not performed in this study because these patients had too few circulating neutrophils for reliable testing before filgrastim treatment. However, previous reports have shown that neutrophils from these patients treated with filgrastim can phagocytose and kill bacteria normally or near normally.³⁹ The substantial reduction in infection-related events observed in the present trial are consistent with these observations and suggest that neutrophils produced in the treated patients possess at least adequate anti-infective activity.

The adverse effects observed in this trial were relatively mild and suggest that filgrastim may be well tolerated as a long-term therapy. Some degree of splenomegaly occurred in many patients, but it generally did not cause symptoms and splenectomies were not required. Animal studies suggest that the splenic enlargement is attributable to extramedullary hematopoiesis owing to mobilization of early hematopoietic

precursors from the marrow to the spleen. Bone pain early in therapy (presumably due to marrow expansion), headache, skin rashes, and thrombocytopenia were generally readily managed and did not require discontinuation of filgrastim therapy.

Another hematopoietic growth factor, granulocyte-macrophage colony-stimulating factor (GM-CSF), has been successfully used to treat chronic neutropenia secondary to myelodysplastic syndromes,⁴³ acquired immunodeficiency syndrome (AIDS),⁴⁴ chemotherapy,⁴⁵ and in patients undergoing autologous BM transplantation.⁴⁶ However, preliminary reports have not demonstrated efficacy of rhGM-CSF in the treatment of severe chronic neutropenia. Welte et al⁴⁰ administered rhGM-CSF to five patients with congenital neutropenia, only one of whom showed an increase in ANC. These five patients were then treated with filgrastim, 3 to 15 $\mu\text{g}/\text{kg}/\text{d}$, and all patients responded with an increase in ANCs to greater than $1.0 \times 10^9/\text{L}$. Differential effects of these hematopoietic growth factors were seen also in one patient with cyclic neutropenia.³⁶ Again, rhGM-CSF treatment failed to augment the ANCs, whereas filgrastim therapy, 1 to 3 $\mu\text{g}/\text{kg}/\text{d}$, significantly increased the ANCs. Further studies are needed to define the basis for the differential effect of filgrastim and rhGM-CSF in treatment of these forms of severe chronic neutropenia. Additional studies are also required to determine if these cytokines are useful therapies for patients with other types of chronic neutropenia, eg, Felty's syndrome and other rheumatologic diseases, symptomatic immune neutropenia, drug-induced agranulocytosis, and the neutropenia associated with glycogen storage disease.

Because the patients in this trial, as well as in those previous phase I/II trials, have benefited substantially from this new therapy, almost all originally treated patients continue to receive filgrastim. Several have been treated for greater than 3 years.⁴⁷ Analysis to date of their treatment responses generally show that neutrophil counts are maintained on therapy, BM examinations are unchanged, "marrow exhaustion" or other untoward hematologic consequence have occurred infrequently, and the clinical benefits noted in this trial have continued. The reduced frequency of fevers, inflammation, and infections has resulted in an improved quality of life for these patients.⁴⁸ However, further long-term follow up is needed to clarify the continued efficacy and safety of this new therapy.

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