Maintenance Treatment of Patients With Myelodysplastic Syndromes Using Recombinant Human Granulocyte Colony-Stimulating Factor

By Robert S. Negrin, Douglas H. Haeuber, Arnon Nagler, Yukio Kobayashi, Jeffrey Sklar, Timothy Donlon, Martha Vincent, and Peter L. Greenberg

Myelodysplastic syndromes (MDS) are characterized by chronic refractory cytopenias resulting in increased risk of infection, bleeding, and conversion to acute leukemia. In an effort to improve these cytopenias we have treated 18 patients over a 6- to 8-week period with increasing daily subcutaneous doses of recombinant human granulocyte colony-stimulating factor (G-CSF). Sixteen patients responded with improvement in neutrophil counts. On cessation of treatment these counts returned to baseline values over a 2- to 4-week period. To maintain these improved blood counts 11 patients were treated with G-CSF for more prolonged periods. Ten patients again responded with an increase in total leukocyte counts (1.6- to 6.4-fold) and absolute neutrophil counts (ANC) (3.6- to 16.3-fold), with responses persisting for 3 to 16 months. A significantly decreased risk of developing bacterial infections was noted during periods with ANC > 1,500/mm^3 as compared with periods of time with ANC < 1,500/mm^3. Two anemic patients had a greater than 20% rise in hematocrit over the study period, and 2 additional patients had a decrease in red blood cell transfusion requirements during G-CSF treatment. Bone marrow myeloid maturation improved in 7 of 9 maintenance phase patients. Three patients progressed to acute myeloid leukemia during treatment. The drug was generally well-tolerated and no severe toxicities were noted. These data demonstrated that G-CSF administered to MDS patients by daily subcutaneous administration was well-tolerated and effective in causing persistent improvement of the neutrophil levels and marrow myeloid maturation. These effects were associated with a decreased risk of infection and, in some patients, with decreased red blood cell transfusion requirements.

THE MYELODYSPLASTIC syndromes (MDS) consist of a group of disorders of hematopoiesis characterized by chronic cytopenias and cytopathies leading to frequent infections, transfusional requirements, and increased risk for conversion to acute myeloid leukemia.[1-6] Treatment options are limited due to the relatively refractory nature of this disease to chemotherapy and the general advanced age of this patient group.[7,8]

The emergence of recombinant human colony-stimulating factors (CSFs) as therapeutic modalities has led to the possibility that these agents may improve the cytopenias that characterize this disease. In vitro studies in MDS have shown the potential of granulocyte-CSF (G-CSF) to enhance myeloid cell differentiation without causing increased clonal self-generation.[9-11] In vivo and in vitro studies in mice have provided evidence for decreased leukemogenicity of leukemic cells after exposure to G-CSF.[12-15] Accordingly, several clinical trials using either G-CSF or granulocyte-macrophage CSF (GM-CSF) have demonstrated that patients with MDS are responsive to these agents and that neutrophil counts can be improved in the majority of patients.[16-20] Our initial phase I/II study demonstrated well-tolerated dose-dependent increases in neutrophil counts in 10 of 12 MDS patients treated with G-CSF over a 6- to 8-week period.[20] In these trials, improvements in blood counts were dependent on continued treatment, as upon cessation of the CSFs blood counts rapidly reverted to baseline values. Therefore, the chronic nature of this disease would require longer-term, perhaps indefinite administration of these agents to attempt to alter the natural history of MDS, ie, diminish infectious risk and transfusional needs as well as alter the risk of conversion to acute leukemia and improve survival.

Little is known about the chronic administration of these drugs in humans. To evaluate the long-term efficacy, tolerance, and toxicity of G-CSF in MDS patients, we describe results of maintenance subcutaneous administration of this drug to such patients for periods up to 16 months.

MATERIALS AND METHODS

Patients. Eighteen patients with MDS were enrolled in the dose-escalation phase, and 11 of these patients received maintenance treatment. Of the seven patients in the short-term study who did not receive maintenance treatment, four had died (two of MDS-related events) and three patients declined further treatment. All of these patients had responded with an increase in absolute neutrophil counts (ANC) during the dose-escalation phase of treatment. Inclusion and exclusion criteria were the same as those described previously.[20] The clinical characteristics of the patients are listed in Table 1. Written informed consent was obtained from all patients according to guidelines established by the Stanford University Human Experimentation Committee.

Study design. The initial dose-escalation phase of this study has been described previously.[20] Patients were treated by subcutaneous injection of G-CSF beginning at 0.1 μg/kg/d and increased every 2 weeks to 0.3, 1.0, and 3.0 μg/kg/d until normalization of the ANC occurred. On completion of the 8-week dose escalation phase, the G-CSF injections were discontinued and a bone marrow examina-

From the Departments of Medicine and Pathology, Stanford University Medical Center; Palo Alto Veterans Administration Medical Center; AMGen, Thousand Oaks, CA; and Division of Diagnostic and Molecular Biology, Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

Submitted November 6, 1989; accepted March 6, 1990.

Supported in part by USPHS Grant CA36915 from the National Cancer Institute, and by AMGen.

*Presented in part at the International Society of Experimental Hematology meeting, August 1989, Paris, France (*Exp Hematol *17:657a, 1989).*

Address reprint requests to Robert S. Negrin, MD, Division of Hematology, Stanford University Medical Center, Room S161, Stanford, CA 94305-5112.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" *in accordance with 18 U.S.C. section 1784 solely to indicate this fact.*

0006-4971/90/7601-0021$3.00/0

Table 1. Profiles of MDS Patients

MDS Classification	Dose-Escalation Phase	Maintenance Phase
RA	2	1
RAEB	9	6
RAEB-T	7	4
Total	18	11
Hematologic characteristics		
Anemia	18	11
Neutropenia		
(<1,800/mm^3)	17	10
(<500/mm^3)	11	5
Thrombocytopenia	15	9
Cytogenetic abnormalities	6	4

Ages: 62 to 78 years; sex: 14 male, 4 female.

Abbreviations: RA, refractory anemia; RAEB, refractory anemia with excess blasts; RAEB-T, refractory anemia with excess blasts in transformation.

tion was performed. Patients were then eligible to restart the G-CSF injections when their blood counts decreased to pretreatment levels, which occurred in all cases. At that time the G-CSF injections were restarted at the dose previously shown to result in normalization of the ANC (between 1,800 and 7,000/mm^3). An attempt was made to maintain the ANC in this range. If the neutrophil count remained less than 1,800/mm^3 the G-CSF was increased to 5.0, and, if needed, to 10.0 μg/kg/d. Complete blood counts were obtained weekly, chemical screening battery every other week, and a urinalysis was obtained monthly. A bone marrow aspiration was performed at the end of 6-month treatment periods or when clinically indicated and sent for morphologic analysis, cytogenetic study, and in vitro culture. Results of in vitro marrow culture studies will be reported separately. In four patients, resting radionuclide cardiac ventriculography to estimate ejection fraction, and spirometry with monthly carbon monoxide diffusion capacity measurements to assess pulmonary function were performed before, during, and after treatment with G-CSF.

Patients were questioned concerning adverse reactions at least weekly, at which time the next week's supply of G-CSF was administered. Patients were instructed to draw up the appropriate amount of G-CSF into a syringe and self-inject the material subcutaneously. Treatment was discontinued if severe toxic reactions occurred or at the patient's request.

Recombinant human G-CSF was provided by AMGen (Thousand Oaks, CA), the composition of which has been described previously.[20] Cytogenetic studies were performed as described.[20] Tests for anti–G-CSF antibody were performed on sera from 10 patients obtained before and during treatment by previously described methods.[21]

Clonal analysis using X-linked restriction fragment length polymorphisms (RFLP) was performed as previously described.[22] Briefly, high molecular weight DNA was extracted from bone marrow and peripheral blood by standard techniques involving sodium dodecyl sulfate (SDS)-proteinase K digestion, phenol extraction, and ethanol precipitation. Each analysis was performed on 10 μg of DNA. A polymorphic *Bgl*II site lying near the pyruvate glycerol kinase (PGK) and hypoxanthine phosphoribosyltransferase (HPRT) genes was found to distinguish active from inactive alleles. After further digestion with *Eco*RI and *Bgl*I to render the polymorphic fragments convenient sizes for analysis, half the DNA was digested with *Hpa*II. The samples were probed with an 800-base pair (bp) *Eco*RI-*Bam*HI fragment of the 5′ end of the PGK gene (gift from Dr B. Vogelstein, Johns Hopkins University, Baltimore, MD). All enzymes were obtained from New England Biolabs, Inc (Boston, MA).

Statistical analysis of the relative risk of infectious episodes was performed by testing the hypothesis that two Poisson events were the same when based on different time intervals. The *P* values were calculated using two-tailed analyses that were corrected for continuity.

RESULTS

Hematologic responses. Eighteen patients were entered into the 6- to 8-week dose-escalation phase of treatment with G-CSF. The short-term hematopoietic responses of the first 12 patients have been reported previously.[20] Sixteen of 18 patients responded with a rise in white blood cells (WBC) (1.9- to 12-fold) and ANC (5- to 40-fold). In all patients blood counts returned to the pretreatment baseline values over 2 to 4 weeks on discontinuation of the G-CSF injections.

Eleven patients enrolled in the long-term maintenance phase of the trial. The hematopoietic reponses of these patients are shown in Table 2 and Fig 1. In this group 10 patients had a rise in WBC (1.6- to 6.4-fold) and ANC (3.6- to 16.3-fold) over baseline pretreatment levels. A positive neutrophil response was defined as being either a normalization of the ANC (eight patients), a rise in ANC to above 1,000/mm^3 if the ANC was initially less than 500/mm^3 (one patient), or a greater than 100% increment if the ANC was initially normal (one patient). These positive responses have been maintained during therapy for periods to date of 6 to 16 months in eight of the patients, and the other two responding patients have been maintained for 3 months (Table 2, Fig 1). The ANC responses of the maintenance phase patients are shown in Fig 1. Five patients began the maintenance phase of treatment with less than 500 neutrophils/mm^3, four responded with increases to greater than 1,300 neutrophils/mm^3.

In six patients the G-CSF injections were discontinued after 6 months as per protocol. In all patients there was a decline in both WBC and ANC toward the pretreatment baseline value (Fig 1). This occurred over a 4- to 8-week period, which was slightly longer than after the initial dose-escalation phase of the trial. Five patients were restarted on the G-CSF (one patient declined retreatment) and again had persistent responses of both the WBC and ANC. In the remaining five patients the G-CSF was continued without interruption. The dose of G-CSF required to maintain these increased neutrophil counts for different patients was variable, ranging from 0.3 to 10 μg/kg/d, although the dose was relatively constant for each patient. The doses required to maintain the neutrophil counts in the normal range are listed in Table 2. Most patients continued to respond to the initial dose used, which resulted in normalization of the neutrophil count during the dose-escalation study that ranged from 1 to 3 μg/kg/d. Patient no. 12 required up to 10 μg/kg/d to normalize the ANC; however, he has been maintained on 5 μg/kg/d for over 6 months. Patient no. 18 initially responded to 3 μg/kg/d with an ANC > 1,800/mm^3; however, after 4 months his ANC dropped to less than 500/mm^3 and the dose was increased to 5 μg/kg/d with a rise in ANC to 1,300/mm^3. There was no apparent relationship between French-American-British (FAB) classification and dose required for a neutrophil response. None of the 10

Table 2. Clinical Features and Hematopoietic Responses of MDS Patients Receiving Maintenance Treatment With G-CSF

Patient No.	Diagnosis*		Dose (μg/kg/d)	Mos of Treatment	WBC × 10^9/L	ANC × 10^9/L	Reticulocytes × 10^9/L	Hematocrit (%)	Platelets × 10^9/L	Blasts × 10^9/L	Marrow Cytogenetics
1	RAEB	Pre			1.8	0.7	17	29.5†	23	.04	NN
		Post	0.5–1.0	16	7.8	6.6	34	30.4†	24	0	NN
3	RAEB	Pre			4.3	3.6	94	39.5	69	0	NN
		Post	1.0	8	14.8	12.7	175	36.7	57	0	NN
6	RAEB	Pre			1.8	0.3	28	27.7†	32	.02	AA‡
		Post	3.0	13	5.4	4.4	25	24.0	83	0	AA‡
10	RAEB	Pre			3.4	1.7	76	29.8	62	0	NN
		Post	1.0	13	11.6	8.8	62	34.7	66	0	NN
12	RA	Pre			1.5	0.4	35	31.0	136	0	NN
		Post	3.0–10.0	11	2.9	1.8	57	33.0	127	0	NN
13	RAEB	Pre			3.7	0.9	84	32.6	59	0	NN
		Post	0.3	10	14.7	11.3	75	37.3	64	0	NN
14	RAEB	Pre			3.7	1.2	34	30.4	313	0	NN
		Post	0.3	10	7.8	4.2	62	32.8	264	0	NN
16	RAEB-T	Pre			0.8	0.05	10	24.0†	20	.05	AN§
		Post	0.3–1.0	6	0.8	0.02	0	25.3†	15	.10	AN§
17	RAEB-T	Pre			4.8	0.4	49	28.7†	67	.91	NN
		Post	0.3	3	30.7	6.5	31	24.1†	30	13.20	AN‖
18	RAEB-T	Pre			1.6	0.2	29	23.6†	27	.08	—
		Post	3–5	8	2.7	1.3	69	29.9†	21	.08	—
19	RAEB-T	Pre			2.5	0.9	17	19.3†	257	.12	AA¶
		Post	3.0	3	8.5	4.8	14	21.0†	286	0	AA¶

Post-values obtained during therapy for the indicated months of treatment.

*RAEB, Refractory anemia with excess blasts; RA, refractory anemia; RAEB-T, refractory anemia with excess blasts in transformation (FAB Classification).

†Transfusion-dependent.

Karyotypes of patients with chromosomal abnormalities. Abbreviations: AA, abnormal chromosomes in all metaphases; AN, mixture of normal and abnormal chromosomes; NN, normal chromosomes.

‡46XY,−7+der7 t(1;7)(p11,q11),del(20)(q11)/47,XY,−7,+8,+der(7)t(1;7)(p11;q11),del(20)(q11).

§46XY,−7+der7 t(1;7)(p11,q11)/46XY.

‖46XY,t(5;16)(q13;p11.2)/46XY.

¶46XX,3q+.

patients tested has developed antibodies to G-CSF, including the two nonresponding patients.

Red blood cell (RBC) responses were more variable. Of the 10 anemic patients treated in maintenance phase, two patients without a transfusion requirement had a greater than 20% increase in hemoglobin levels (Table 2, patient nos. 10 and 13). Two more severely anemic individuals (patient nos. 1 and 6) had decreases in transfusion requirements.

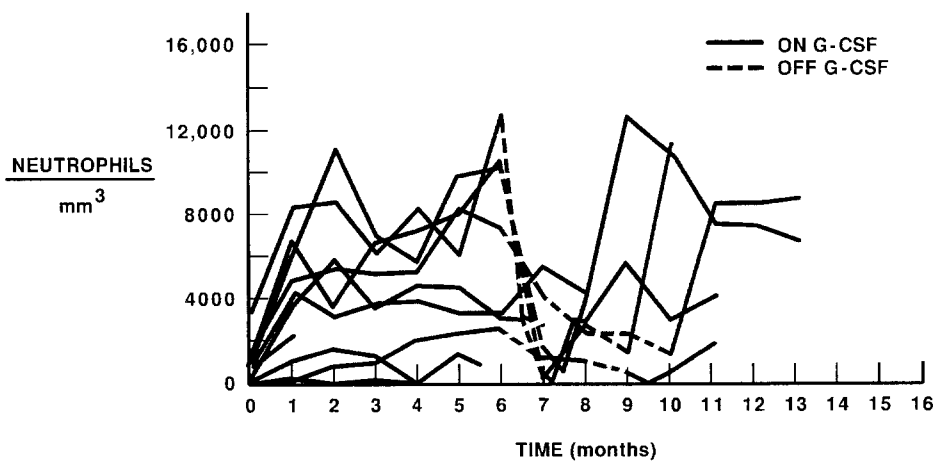

Fig 1. Neutrophil responses of maintenance phase MDS patients to G-CSF. Absolute neutrophil counts are plotted for all maintenance phase patients. The solid and broken lines denote periods of time on and off G-CSF therapy, respectively.

Patient no. 1 initially required 3 to 4 U of RBCs every 4 to 6 weeks, which temporarily decreased to 5 U during 6 months of treatment concomitant with doubling of his reticulocyte count. In patient no. 6, RBC requirements decreased from 2 to 4 U of RBCs every 4 to 6 weeks before G-CSF treatment to 4 U of RBCs during 13 months of G-CSF treatment. Platelet counts generally remained stable during the treatment period; however, patient no. 6 had an increase in platelet count from 32,000 to 83,000/mm^3 and patient no. 17 had a decrease in platelet count from 67,000 to 30,000 (Table 2). There were no significant changes in monocyte, lymphocyte, basophil, or eosinophil counts during treatment.

Six patients had circulating myeloblasts at the beginning of the maintenance phase (Table 2). After several weeks of treatment these cells were no longer detected in four individuals (patient nos. 1, 6, 18, and 19). Three patients (nos. 1, 16, and 17, one initially with RAEB and two with RAEB-T, respectively) progressed to AML during the maintenance phase after 16, 3, and 6 months of treatment, and died 1, 2, and 8 months later, respectively.

Bone marrow morphology was evaluated in nine patients before the study, after the dose-escalation phase, and after 6 months of maintenance treatment. In seven responding patients there was improved marrow myeloid maturation, with a decrease in the number of myeloblasts and an increase in the number of neutrophils (Table 3). To further quantitate these findings a relative myeloid differentiation index was calculated. This index was defined as the percentage of neutrophils, myelocytes, and metamyelocytes divided by the percentage of myeloblasts and promyelocytes. There was an increase in this index, indicating enhanced marrow myeloid cell maturation, in the seven responding patients after both the dose-escalation phase and 6 months of maintenance treatment (Fig 2, Table 3).

Cytogenetic analysis was performed on marrow cells from 10 maintenance phase patients; 7 of whom had all normal karyotypes, 1 had a mixture of normal and abnormal chromosomes (AN), and 2 of whom (patient nos. 6 and 19) had all abnormal metaphases (12 and 3 metaphases, respectively) at the beginning of the study (Table 2). In addition, patient no. 7, who completed only the dose-escalation study, had complex karyotypic abnormalities including a del (5)(q13,p33) in all 21 metaphases before treatment with G-CSF.[20] All of these patients, except for the AN patient, responded with increased ANCs while on G-CSF (reference 20, Table 2). In responding patient nos. 7 and 19, the initial cytogenetic abnormalities persisted (19 and 8 metaphases, respectively) after the dose-escalation phase of treatment. Patient no. 6 developed one normal metaphase out of seven after the dose-escalation phase. This patient had additional cytogenetic studies after 6 and 12 months of maintenance treatment in which the same abnormal clone was found in 19 of 20 metaphases in both studies. A fourth individual (patient no. 17), initially with all normal cytogenetics, developed a mixture of normal (15) and abnormal (3) metaphases during treatment.

To further analyze the issue of the clonal nature of the responsive cells, RFLP analysis was performed on two X-linked genes (PGK and HPRT) using DNA from the four female patients. Three individuals were not polymorphic at these genes, whereas one individual (patient no. 7) was polymorphic at the PGK gene. After triple digestion of this patient's DNA with *Bgl*I, *Bgl*II, and *Eco*RI, two bands appeared on Southern blot autoradiograms using the PGK probe at 1.7 and 1.3 kilobases ([kb], Fig 3). After digestion with the methylation sensitive enzyme *Hpa*II, the 1.3-kb band disappeared in all bone marrow and blood cell populations, indicating clonal hematopoiesis before treatment. After treatment with G-CSF, in which this patient responded with an increase in ANC from 1,100/mm^3 to 5,600/mm^3,[20] an identical clonal pattern was observed in both the mononuclear cell and neutrophil fractions (Fig 3).

Infectious episodes. In four maintenance phase patients there were eight episodes of clinically significant bacterial infections after beginning the G-CSF injections (Table 4). These infections were defined as either having positive cultures or a source of infection identified and being signifi-

Table 3. Bone Marrow Morphology of Maintenance Phase Patients With MDS

Patient No.	Time*	Myeloblasts (%)	Neutrophils (%)	Cellularity (%)	Myeloid: Erythroid Ratio	RMDI
1	Before	9	3	30	0.5	0.4
	DE	1	25	60	1	2.3
	M	11	38	60	4	1.8
3	Before	6	19	60	2	1.7
	DE	4	49	60	4	3.0
	M	2	46	70	3	4.4
6	Before	14	15	45	1.5	1.1
	DE	2	38	65	3	3.4
	M	4	42	50	4	2.8
10	Before	19	15	40	2.5	0.9
	DE	6	36	55	3.5	2.2
	M	2	47	45	5	4.7
12	Before	6	11	30	1	1.1
	DE	11	19	50	2	1.5
	M	2	28	70	2.5	2.3
13	Before	5	33	ND	4	2.6
	DE	4	47	ND	4	9.1
	M	4	50	75	7	4.3
14	Before	10	34	ND	4	2.7
	DE	6	39	ND	4	5.3
	M	4	40	70	5	4.7
16	Before	30	11	40	5	0.5
	DE	42	6	40	5	0.2
	M	55	7	40	10	0.2
17	Before	22	29	ND	4	1.4
	DE	32	17	ND	3	0.6
19	Before	17	11	ND	1.5	0.9
	DE	10	22	70	2	1.7

Abbreviations: RMDI, relative myeloid differentiation index; ND, not done.

*Time points: Before, before treatment; DE, after the dose-escalation period; M, after 6 months of maintenance treatment.

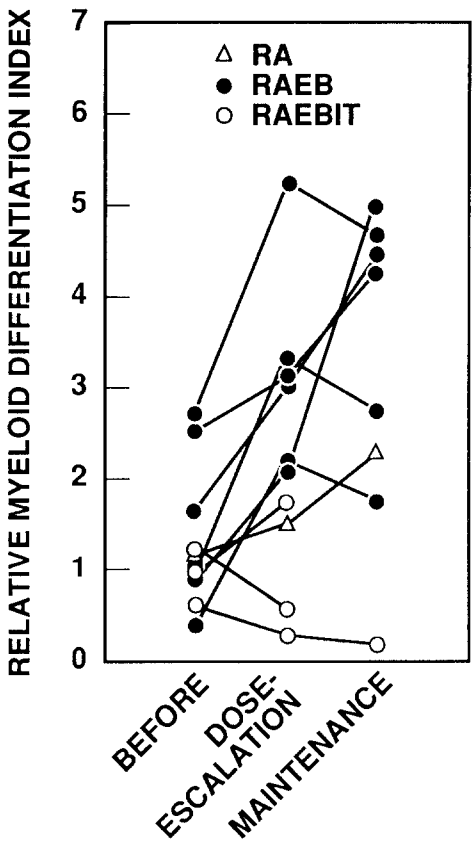

Fig 2. Myeloid marrow cell response of MDS patients to G-CSF treatment. The relative myeloid differentiation index (defined as neutrophils + metamyelocytes + myelocytes ÷ promyelocytes + myeloblasts) calculated from differential counts of bone marrow aspirates is shown. Aspirations were performed before study entry, at the end of the dose-escalation phase, and during maintenance treatment.

cant enough to require admission to the hospital for treatment with intravenous antibiotics. Of these infectious episodes, seven occurred at times when the ANC was less than 1,500/mm^3 either before a patient responded or between treatment cycles. As shown in Table 4, these seven infections developed during the 44 months of observation when the ANC was less than 1,500/mm^3, corresponding to an infection risk of 0.16 episodes per month. For the 10 neutropenic patients who responded to the G-CSF injections and achieved an ANC > 1,500/mm^3 during 95 months of observation, only 1 infectious episode occurred, indicating a significantly reduced infection risk of 0.01 episodes per month ($P < .006$). Retrospective analysis of these 10 patients' records for a period of time similar to their respective treatment periods resulted in an additional 100 months of observation before entering the study. During this pretreatment period there were an additional five infectious episodes, all at times when the ANC was less than 1,500/mm^3 for an infection risk of 0.05 episodes per month (Table 4). When these data are included in the analysis of patients with ANC < 1,500/mm^3, this infection risk remains significantly more than that of patients who achieved an ANC > 1,500/mm^3 with G-CSF treatment (0.08 compared to 0.01 episodes per month, $P < .04$).

Toxicity. Relatively little toxicity was associated with the chronic use of G-CSF. There were no infections or rashes at the injection sites, although local bruising was noted on occasion in two thrombocytopenic patients. Bone pain was not reported. Several patients had fever over the course of the study. However, in most cases this resolved over 1 to 3 days, consistent with a viral-like illness. In our previous dose-escalation trial several patients with preexisting cardiac and pulmonary disease had these clinical problems during G-CSF treatment.[20] Although it was not felt that these episodes were related to the G-CSF, cardiac radionuclide ventriculograms and pulmonary function with diffusion studies were performed on four patients at the beginning, during, and after 6 months of maintenance treatment to evaluate the impact of G-CSF on cardiac and pulmonary function. No changes were noted in cardiac ejection fraction, lung diffusion capacity, or pulmonary function in these patients (data not shown).

DISCUSSION

In this report we have evaluated relatively long-term tolerance, efficacy, and toxicity of G-CSF in MDS patients, extending our initial observations on the short-term efficacy of this treatment.[20] Ten of 11 maintenance-phase MDS patients had persistent improvements in neutrophil counts for periods up to 16 months (8 patients have responded for greater than 6 months to date). Continued treatment was necessary to maintain improved ANCs as blood counts reverted to the pretreatment baseline values over 4 to 8 weeks after stopping the drug.

Improvement in marrow myeloid maturation, as quantitated by the relative myeloid differentiation index, was also demonstrated in responding patients. This was noted in all six of the patients with the subclassification of RAEB, and 1 of the 3 RAEB-T patients evaluable for morphologic assessment. The other two RAEB-T patients had decreased myeloid maturation concomitant with their progression to AML. In this regard, combined data of MDS patients treated with GM-CSF indicate that 7 of 45 patients have progressed to AML in five short-term studies, particularly in those individuals with greater than 14% marrow blasts.[16-19,23] Our present study indicates that this phenomenom can also be observed during treatment with G-CSF, as 3 of 18 patients converted to AML over the course of treatment. Nine of our patients had greater than 14% marrow blasts at study entry (reference 20, Table 3), two of whom progressed to AML during G-CSF treatment. Because evolution to AML is part of the natural history of this disease, randomized controlled trials will be needed to determine whether such treatment with CSFs alters this predisposition.

Cytogenetic abnormalities, when present, persisted after G-CSF treatment. In patient no. 6 abnormal metaphases were noted at study entry, after the dose escalation phase and after 6 and 12 months of maintenance treatment. This

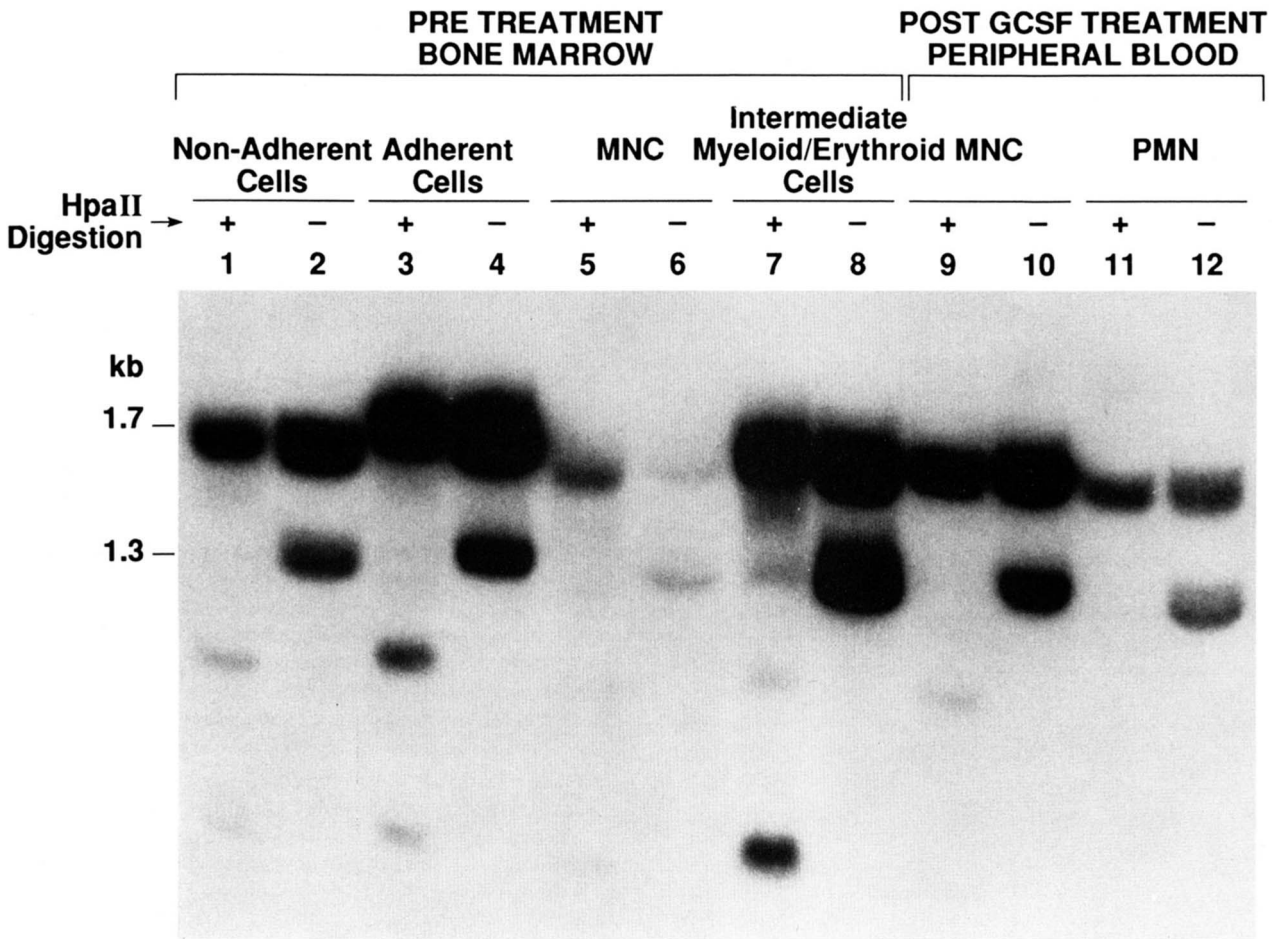

Fig 3. RFLP analysis of clonality. Pretreatment bone marrow cells from patient no. 7 were separated into nonadherent, adherent, mononuclear (MNC), and intermediate myeloid/erythroid fractions. Posttreatment peripheral blood was separated into MNC and neutrophil (PMN) fractions. After preparation of DNA, restriction fragments were generated and half of the sample was further digested with the methylation sensitive enzyme *Hpa*II. The samples were probed with an 800-bp fragment from the 5′ end of the PGK gene. All pretreatment bone marrow fractions show 1.7- and 1.3-kb fragments when not subjected to digestion with *Hpa*II (lanes 2, 4, 6 and 8), whereas only the 1.7-kb fragment remains after digestion with *Hpa*II (lanes 1, 3, 5, and 7), indicating clonality of the cells. After treatment with G-CSF, identical patterns are seen using peripheral blood mononuclear cells (MNCs) and neutrophils (PMNs, lanes 9 through 12).

patient had a dramatic and persistent rise in neutrophil count while on G-CSF. Two other patients, both with all abnormal metaphases, also had persistence of these abnormalities after responding to G-CSF with an increase in ANC. These data suggested that differentiation of the abnormal clone occurred with treatment. Further support for the induced differentiation of the abnormal clone by G-CSF was obtained using RFLP analysis of X-linked genes. Using this type of evaluation, clonal hematopoiesis has previously been demonstrated in approximately 35% of patients with MDS.[24] One of four responding female patients was polymorphic at the PGK gene locus and the clonal nature of her neutrophils was demonstrated before and after treatment with G-CSF (Fig 3). These data indicate that the neutrophil response in this MDS patient treated with G-CSF was due to maturation of the abnormal clone rather than stimulation of residual normal hematopoiesis. This result is in contrast to the finding of polyclonal hematopoiesis in one recently reported patient analyzed in this fashion who was treated with GM-CSF.[25] Other patients will need to be evaluated in this way to determine the proportion of patients with clonal responses after growth factor treatment.

In retrospective analysis there was a significant reduction in bacterial infection risk during periods with an ANC > 1,500/mm^3 with G-CSF therapy, as compared to periods with an ANC < 1,500/mm^3 (Table 4). We previously showed that in vitro neutrophil function in MDS patients after G-CSF therapy demonstrated enhanced phagocytosis and maintained chemotaxis in the majority of patients.[20] These data indicate that in addition to increasing neutrophil counts, G-CSF treatment was associated with possible clinical efficacy. However, an apparent although statistically insignificant increase in infection risk was noted in patients who were treated with G-CSF but had not yet achieved an ANC > 1,500/mm^3 (Table 4, A *v* B). This may be merely a reflection of the small number of infectious episodes that

Table 4. Relative Risk of Bacterial Infections in MDS Patients

	Before G-CSF Treatment	On G-CSF Protocol	
	Neutrophil Count/mm³	Neutrophil Count/mm³	
	≤1,500	≤1,500	>1,500
	A	B	C
Patients with infections	5	4	1
Episodes of infections	5	7	1
Months of risk*	100	44	95
Infections/mo	0.05	0.16	0.01

Variables	*P* Values†
A + B *v* C	.04
B *v* C	.006
A *v* C	NS
A *v* B + C	NS
A *v* B	NS

Abbreviations: A, patients 6, 12, 16, 18, and 19 had infections before G-CSF therapy; B, patients in this group had either not yet had neutrophil responses (early in treatment), or had decreased neutrophil levels after discontinuing G-CSF as per protocol; C, patients in this group had persistent neutrophil responses to G-CSF.

*Ten neutropenic patients were retrospectively evaluated for 2 to 15 months before treatment and during 4 to 16 months of treatment with G-CSF.

†*P* Values for the relative risk of infections were calculated as a test for differences between two Poisson variables, corrected for continuity, two-tailed.

occurred, the more advanced state of the patient's disease at the time of study entry, or an adverse effect of G-CSF on neutrophil activity immediately after initiating therapy and before elevation of the neutrophil count. To rigorously demonstrate that G-CSF treatment-associated improved neutrophil counts result in protection of these individuals from infections, prospective clinical trials will be needed.

Our patients experienced a low incidence of mild side effects, indicating good tolerance to long-term administration of G-CSF. All patients learned to self-administer the drug subcutaneously as outpatients. No patient developed antibodies to G-CSF over the course of treatment.

These data suggest that long-term maintenance treatment of MDS patients with G-CSF is well-tolerated, and results in persistent improvement in neutrophil counts in the majority of patients, and increased hemoglobin levels in some patients. In addition, in retrospective analysis a reduced incidence of infections was observed in responding patients.

ACKNOWLEDGMENT

The authors thank Dana Bangs; Jan DiJulio, RN, MSN; Maureen O'Hara, RN; Claudia Rupp, PharmD; Byron Brown, PhD; Valerie Cannon; Jane Pelton; and Shirley Gray, Stanford University Medical Center, for expert assistance. The superb care given by the nursing staff on the Clinical Research Center of Stanford University Hospital is also greatly appreciated.

REFERENCES

1. Block M, Jacobson LO, Bethard WF: Preleukemic acute leukemia. JAMA 152:1018, 1953

2. Linman JW, Bagby GC Jr: The preleukemic syndrome (hematopoietic dysplasia). Cancer 42:854, 1978

3. Greenberg PL: The smoldering myeloid leukemic states: Clinical and biologic features. Blood 61:1035, 1983

4. Pierre RV: Preleukemic states. Semin Hematol 11:73, 1974

5. Cohen JR, Creger WP, Greenberg PL, Schrier SL: Subacute myeloid leukemia: A clinical review. Am J Med 66:959, 1979

6. Tricot G, Mecucci C, Van den Berghe H: Evolution of the myelodysplastic syndromes. Br J Haematol 63:609, 1986

7. Mertelsmann R, Tzvi Thaler H, To L, Gee TS, McKenzie S, Schauer P, Friedman A, Arlin Z, Cirrincione C, Clarkson B: Morphological classification, response to therapy, and survival in 263 adult patients with acute nonlymphoblastic leukemia. Blood 56:773, 1980

8. Armitage JO, Dick FR, Needelman, SW, Burns CP: Effect of chemotherapy for the dysmyelopoietic syndrome. Cancer Treat Rep 65:601, 1981

9. Nagler A, Ginzton N, Negrin RS, Bangs C, Donlon T, Greenberg PL: In vitro effects of recombinant human granulocyte and granulocyte-monocyte colony stimulating factors on hemopoiesis in the myelodysplastic syndromes. Leukemia 4:193, 1990

10. Nagler A, Ginzton N, Negrin RS, Donlon T, Souza LM, Greenberg PL: In vitro hemopoiesis in myelodysplastic syndrome patients treated with recombinant human granulocyte colony stimulating factor. Blood 72:128a, 1988 (abstr)

11. Nagler A, Negrin RS, Ginzton N, Bangs D, Donlon T, Greenberg PL: Impact of marrow cytogenetics and morphology on in vitro hemopoiesis in myelodysplastic syndromes (MDS). Exp Hematol 17:646a, 1989 (abstr)

12. Metcalf D, Nicola NA: Autoinduction of differentiation in WEHI-3B leukemia cells. Int J Cancer 30:773, 1982

13. Lotem J, Sachs L: In vivo inhibition of the development of myeloid leukemia by injection of macrophage and granulocyte-inducing protein. Int J Cancer 28:375, 1981

14. Lotem J, Sachs L: Control of in vivo differentiation of myeloid leukemic cells. IV. Inhibition of leukemia development by myeloid differentiation-inducing protein. Int J Cancer 33:147, 1984

15. Begley CG, Metcalf D, Nicola NA: Purified colony stimulating factors (G-CSF and GM-CSF) induce differentiation in human HL60 leukemic cells with suppression of clonogenicity. Int J Cancer 39:99, 1987

16. Vadhan-Raj S, Keating M, LeMaistre A, Hittelman WN, McCredie K, Trujillo JM, Broxmeyer HE, Henney C, Gutterman JU: Effects of recombinant human granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndromes. N Engl J Med 317:1545, 1987

17. Ganser A, Volkers B, Greher J, Ottmann OG, Walther F, Becher R, Bergmann L, Schulz G, Hoelzer D: Recombinant human granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndromes. A phase I/II trial. Blood 73:31, 1989

18. Antin JH, Smith BR, Holmes W, Rosenthal DS: Phase I/II study of recombinant human granulocyte-macrophage colony-stimulating factor in aplastic anemia and myelodysplastic syndrome. Blood 72:705, 1988

19. Thompson JA, Lee DJ, Kidd P, Rubin E, Kaufmann J, Bonnem EM, Fefer A: Subcutaneous granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndrome: Toxicity, pharmacokinetics and hematologic effects. J Clin Oncol 7:629, 1989

20. Negrin RS, Haeuber DH, Nagler A, Olds LC, Donlon T, Souza LM, Greenberg PL: Treatment of myelodysplastic syndromes with recombinant human granulocyte colony-stimulating factor. A phase I/II trial. Ann Int Med 110:976, 1989

21. Tsu T, Herzenberg LA: Solid-phase radioimmune assays, in Mishell BB, Shiigi SM (eds): Selected methods in cellular immunogy. San Francisco, CA, Freeman, 1980, p 373

22. Vogelstein B, Fearon ER, Hamilton SR, Preisinger AC, Willard HF, Michelson AM, Riggs AD, Orkin SH: Clonal analysis using recombinant DNA probes from the X-chromosomes. Cancer Res 47:4806, 1987

23. Herrmann F, Lindemann A, Klein H, Lubbert M, Schulz G, Mertelsmann R: Effect of recombinant granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndrome with excess blasts. Leukemia 3:335, 1989

24. Janssen JWG, Buschle M, Layton M, Drexler NG, Lyons J, Van den Berghe H, Heimpel H, Kubanek B, Kleihauer E, Mufti GJ, Bartram CR: Clonal analysis of myelodysplastic syndromes: Evidence of multipotent stem cell origin. Blood 73:248, 1989

25 Vadhan-Raj S, Broxmeyer HE, Spitzer G, LeMaistre A, Hultman S, Ventura G, Tigaud J-D, Cork MA, Trujillo JM, Gutterman JU, Hittelman WN: Stimulation of nonclonal hematopoiesis and suppression of the neoplastic clone after treatment with recombinant human granulocyte-macrophage colony-stimulating factor in a patient with therapy-related myelodysplastic syndrome. Blood 74:1491, 1989