Use of Recombinant Human Granulocyte-Macrophage Colony-Stimulating Factor in Graft Failure After Bone Marrow Transplantation

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The effect of recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) was evaluated in 37 patients with marrow graft failure after allogeneic (n = 15), autologous (n = 21), or syngeneic (n = 1) bone marrow transplantation. rhGM-CSF was administered by 2-hour infusion at doses between 60 and 1,000 $\mu g/m^2/d$ for 14 or 21 days. At doses of less than 500 $\mu g/m^2$, rhGM-CSF was well-tolerated and did not exacerbate graft-versus-host disease in allogeneic transplant recipients. No patient with myelogenous leukemia relapsed while receiving rhGM-CSF. Twenty-one patients reached an absolute neutrophil count (ANC) $\geq 0.5 \times 10^9/L$ within 2 weeks of starting therapy while 16 did not. None of seven patients who received chemically purged autologous marrow grafts responded to rhGM-CSF. The survival rates of GM-CSF–treated patients were significantly better than those of a historical control group.

ALTHOUGH SUSTAINED engraftment with recovery of hematopoiesis to normal levels is the general rule after marrow transplantation, some patients fail to engraft and others develop graft failure after temporary engraftment. Two broad categories of graft failure have been recognized. One category, termed graft rejection, occurs after allogeneic transplantation and is characterized by the regrowth of immunocompetent host cells and a simultaneous loss of donor cells. Immunologically mediated graft rejection is seen in only about 2% of marrow graft recipients after cyclophosphamide/total body irradiation preparative regimens when unmanipulated HLA-matched donor marrow is used.[1] However, in partially mismatched donor transplants,[2,3] T-cell–depleted marrow transplants,[4] or bone marrow transplants (BMT) for aplastic anemia (AA),[5,6] graft rejection is more common. The second broad category of graft failure applies to those patients who have no evidence for regrowth of host immunocompetent cells but in whom the transplanted marrow nonetheless fails to function normally. Graft failure without evidence of immunologic rejection occurs in as many as 9% of patients after HLA-matched allogeneic BMT for hematologic malignancy,[2] and in a similar percentage of patients after autologous BMT.[7] Potential causes of poor graft function may include a low stem cell innoculum,[8] posttransplant infections with cytomegalovirus,[9] or drug toxicity.[10-12]

Therapy for graft rejection or failure has been either supportive care or a second marrow transplant. With either approach, the prognosis after allogeneic BMT is poor.[5,13-16] With supportive care alone, some patients may spontaneously recover marrow function, but this is unusual. For example, in a recent report from the International Bone Marrow Transplant Registry, 19 patients given allogeneic BMTs for AA failed to reach an absolute neutrophil count (ANC) of 0.100×10^9 per liter by day 28 posttransplant. All of these patients eventually died of complications of pancytopenia.[15] Continued supportive care for patients with leukemia who fail to engraft yields similarly poor results. Among 86 patients who underwent autologous BMT for lymphoid malignancy in Seattle, WA, 14 did not reach an ANC of $0.100 \times 10^9/L$ by day 28 after marrow infusion. The survival of these 14 patients was 35% at day 100 and 0% at 1 year (unpublished data, October 1988). This differs from survival rates of 65% at day 100 and 35% at 1 year in the 72 patients with adequate engraftment.[7] Second marrow transplants may salvage some patients with graft failure. However, patients with hematologic malignancies given second marrow transplants for graft failure had a 17% long-term survival.[13]

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a regulatory glycoprotein necessary for survival, proliferation, and maturation of myeloid cells, and also enhances the functional activities of neutrophils.[17] Clinical trials have suggested that GM-CSF can accelerate hematopoietic recovery after autologous BMT.[18,19] Because the morbidity and mortality of patients with graft failure are frequently related to neutropenic complications and second marrow grafts or infusions are relatively ineffective, recombinant human GM-CSF (rhGM-CSF) was offered to patients with graft failure to determine its toxicities and its possible efficacy at stimulating hematopoiesis in this setting.

MATERIALS AND METHODS

Patient selection. Patients with malignancy or aplastic anemia who underwent allogeneic, autologous, or syngeneic BMT and subsequently developed graft failure were eligible for the study. Graft failure was defined as failure to achieve an ANC of $0.100 \times 10^9/L$ by day 28 post BMT, failure to achieve an ANC of $0.100 \times 10^9/L$ by day 21 post BMT with a documented life-threatening

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infection, or the loss of engraftment with a mean ANC of less than 0.500×10^9/L for at least 1 week after having initially achieved a mean ANC $\geq 0.500 \times 10^9$/L for 1 week. Allografted patients with immunologic rejection were eligible. Patients with leukemic relapse or potentially reversible pharmacologic causes of graft failure were excluded. No exclusions were made for pretransplant disease type or stage, prior therapy, or Karnofsky performance scores. Signed informed consent conforming to federal drug administration and institutional review guidelines was required.

Clinical monitoring. All patients were examined and had routine blood studies daily. Bone marrow aspirates and biopsies were done within 3 days of therapy and 14 days after starting rhGM-CSF. Samples were sent for histologic analysis, cytogenetic analysis, and, when appropriate, for Y chromosome in situ hybridization studies (D. Durnam, manuscript in preparation) or restriction fragment length polymorphism (RFLP) studies.[20]

Study design. The study was designed as a phase I/II dose-escalation trial in which patients were enrolled consecutively. The dose range of rhGM-CSF[21] (Immunex/Hoechst/Behring, Seattle, WA; yeast-derived; specific activity = 5×10^7 colony-forming units/mg) was 60 to 1,000 $\mu g/m^2$/d. rhGM-CSF was mixed in 50 mL of normal saline with 0.1% albumin and administered via central venous catheter as a single 2-hour intravenous infusion daily for 14 or 21 days. If, 2 weeks after the treatment course, the ANC remained less than 0.500×10^9/L and there was no life-threatening toxicity from the rhGM-CSF and no evidence of leukemic relapse, a second course of treatment at twice the prior dose was administered. A maximum of three courses of rhGM-CSF was administered to each patient. The first five patients on this study received 60 $\mu g/m^2$/d. The starting dose was doubled for subsequent groups of five until toxicity possibly related to rhGM-CSF was identified at 500 $\mu g/m^2$/d. Thereafter, all patients were treated at 250 $\mu g/m^2$/d. If an ANC $\geq 10.0 \times 10^9$/L was reached before completion of rhGM-CSF infusions, it was discontinued.

Patients treated with rhGM-CSF. Fifteen patients with graft failure after allogeneic BMT were studied. The clinical data, type of transplant, conditioning regimen, and institution are shown in Table 1. The mean age was 22 years (range 4 to 51). The mean ANC before starting rhGM-CSF was $0.153 \pm 0.140 \times 10^9$/L (range 0 to 0.360×10^9/L).

Twenty-one patients with graft failure after autologous and one after a syngeneic BMT were treated. Clinical data, the conditioning regimen, and type of marrow purging are given in Table 2. The mean age was 29 years (range 3 to 65). The mean ANC before starting rhGM-CSF was $0.104 \pm 0.130 \times 10^9$/L (range 0 to 0.472×10^9/L).

Measurement of response. A response was defined as an increase in the ANC to $\geq 0.500 \times 10^9$/L within 14 days of starting the final course of rhGM-CSF. If this was not achieved or the patient died before completion of the rhGM-CSF course, the patient was considered as a nonresponder.

Retrospective control patients. To provide an estimate of the effect of graft failure on survival after BMT, the records of 1,679 patients transplanted consecutively at the Fred Hutchinson Cancer Research Center (FHCRC) (Seattle, WA) from January 5, 1981 to May 27, 1987 were reviewed. One-hundred fifty-five patients fulfilled the criteria for graft failure as defined above. The time period for selection of retrospective control cases began with the first case of 1981 and ended at the time of initiation of phase I-II trials of rhGM-CSF in autologous BMT patients.

RESULTS

Response of patients with graft failure after allogeneic BMT. Nine of 15 patients with graft failure after alloge-

Table 1. Characteristics of Patients Treated With rhGM-CSF for Allogeneic Marrow Graft Failure

UPN*	Disease/State†	Age/Sex‡	Type of Host Donor Match§	Conditioning Regimen‖
1001	AA	4 F	Identical	Cy
4421	AA	11 F	Identical	Cy
4402	CML/AP	7 M	Unrelated, one-antigen mismatch	Cy/TBI
BO99	ANL/Rem	27 F	Identical¶	Cy/TBI
4588	ANL/Rem	51 M	Identical	Cy/TBI
4321	CML/BP	32 M	Two-antigen mismatch	Bu/Cy/ATG
4602	ALL/Rel	21 M	Identical	Cy/TBI
4628	HD/Rel	35 M	Identical	Cy/VP-16/BCNU
4631	ANL/Rel	33 M	One-antigen mismatch	Cy/TBI
4594	CML/CP	22 M	Unrelated, matched	Cy/TBI
4599	ALL/Rel	12 M	Identical	Cy/TBI
NY97	ANL/Rem	12 M	Identical¶	Cy/TBI
FL96	ALL/Rel	4 M	Identical	Ara-C/TBI
OS95	NHL/Rem	36 F	Identical	BU /Cy
4391	ALL/Rel	27 M	Unrelated, matched	Cy/TBI

*UPN, unique patient number. Patients treated at FHCRC included 1001, 4421, 4402, 4588, 4321, 4602, 4628, 4631, 4594, 4599, and 4391. Other centers included: Dana Farber Medical Center (BO99), Memorial Sloan Kettering Hospital (NY97), Ohio State University Medical Center (OS95), and University of Florida/Shards Hospital (FL96).

†AA, aplastic anemia; CML, chronic myelogenous leukemia; ANL, acute nonlymphocytic leukemia; HD, Hodgkin's Disease; NHL, non-Hodgkin's lymphoma; ALL, acute lymphocytic leukemia; Rem, remission; Rel, relapse or refractory disease; CP, chronic phase; AP, accelerated phase; BP, blast phase.

‡M, male; F, female; age expressed in years.

§Identical, genotypically HLA identical from a sibling donor; two-antigen mismatch, HLA haploidentical with a 2 HLA loci mismatch on the unshared haplotype from a parent donor; unrelated, matched, phenotypically HLA identical from an unrelated donor; unrelated, one-antigen mismatch, phenotypically identical on 3 HLA loci and 1 nonidentical HLA loci from an unrelated donor.

‖Cy, cyclophosphamide; TBI, total body irradiation; ATG, antithymocyte globulin; Bu, busulfan; VP-16, etoposide; Ara C, cytarabine; BCNU, carmustine.

¶T-cell-depleted marrow.

Table 2. Characteristics of Patients Treated With rhGM-CSF for Autologous or Syngeneic Graft Failure

UPN*	Disease/State†	Age/Sex‡	Conditioning Regime§	Marrow Purging‖
BO98	NHL/Rel	43 F	Cy/TBI	B1
4319	CML/CP	44 F	Cy/ATG¶	—
3958	ANL/Rel	3 F	Cy/TBI	4-HC
OM99	NHL/Rel	3 F	Th/TBI	—
SF96	NEU/Rel	3 M	Chem/TBI	MAN
4050	ALL/Rem	40 M	Cy/TBI	B1
4040	ANL/Rem	22 M	Cy/TBI	4-HC
4325	ANL/Rel	18 M	Cy/TBI	4-HC
4570	NHL/Rel	53 M	Bu/Cy/VP-16	B1
4501	NHL/Rel	46 F	Cy/TBI	B1
OM91	BrCa/R	44 F	Cy/Th/Hu	—
HO90	HD/Rel	30 M	Cy/VP-16/BCNU	—
SC87	ANL/Rem	38 F	Bu/Cy	—
GT86	HD/Rel	18 F	Cy/VP-16/BCNU/CP	—
UC85	ANL/Rem	33 M	Bu/Cy	4-HC
UT84	ALL/Rel	5 M	Bu/Cy	VP-16
SJ83	ANL/Rem	15 M	Bu/Cy	4-HC
OM94	OvCa/Rel	40 F	Cy/TBI	—
MI79	NHL/Rel	65 M	Cy/VP-16/BCNU	—
4721	NHL/Rel	22 M	Cy/TBI	B1
OM81	HD/Rel	25 F	Cy/VP-16/BCNU	—
SK80	Neu/Rel	5 F	Th/CP/BCNU	4-HC

*UPN, unique patient number. Patients treated at FHCRC included: 4319, 3958, 4050, 4040, 4325, 4570, 4501, and 4721. Other centers included: Nebraska University Medical Center (OM99, OM94, OM91, OM81), University of California, San Francisco (SF96), Dana Farber Medical Center (BO98), Michigan University Hospital (MI79), Tulane University Medical Center (UT84), Memorial Sloan Kettering Hospital (SK80), City of Hope National Medical Center (HO90), Georgetown University Medical Center (GT86), Scripps Clinic (SC87), University Hospital of Cleveland (UC85), and St Jude Children's Research Hospital (SJ83).

†CML, chronic myelogenous leukemia; ANL, acute nonlymphocytic leukemia; NHL, non-Hodgkin's lymphoma; ALL, acute lymphocytic leukemia; HD, Hodgkin's disease; Rem, remission; Rel, relapse or refractory disease; CP, chronic phase; NEU, neuroblastoma; Mel, melanoma; BrCa, breast cancer; OvCa, ovarian cancer.

‡M, male; F, female; age expressed in years.

§Cy, cyclophosphamide; TBI, total body irradiation; Th, thiotepa; Chem, VM26, cis platinum (CP), adriamycin, melphalan; HU, hydroxyurea; PM, phenylalanine mustard.

‖B1, B1 monoclonal antibody; 4-HC, 4 hydroxycyclophosphamide; MAN, monoclonal antibody to neuroblastoma.

¶Cyclophosphamide and ATG (anti-thymocyte globulin) were administered as a preparative regime for a second syngeneic marrow infusion. The patient also received nonirradiated granulocyte transfusions from day 2 to day 8 of GM-CSF therapy.

neic BMT increased their ANC to $\geq 0.500 \times 10^9$/L within 14 days of starting rhGM-CSF. Of the nine responders, seven responded after the first course of therapy and two required a second course (see Table 3). The ANC of the 15 patients increased from a mean value of $0.153 \pm 0.140 \times 10^9$/L (0 to 0.360×10^9/L) at the start of treatment to a mean of $2.545 \pm 3.944 \times 10^9$/L (0 to 11.970×10^9/L) on the last day of the final course ($P = .03$; paired t-test). The increase in ANC first occurred between days 4 and 9 after initiation of rhGM-CSF in seven of the responding patients, while two patients (unique patient numbers [UPNs] 4628 and 4594) had an ANC increase within 48 hours. Both of these patients reached ANCs greater than 10.0×10^9/L within 8 days of starting rhGM-CSF.

Eight of nine responders maintained ANCs $\geq 0.500 \times 10^9$/L after discontinuation of rhGM-CSF therapy. One of the nine responders (OS95) had a decrease in ANC to 0.304×10^9/L 14 days after discontinuation of rhGM-CSF. However, she recovered and has adequate hematopoiesis 330 days post BMT (see Table 3).

Six patients did not respond to rhGM-CSF. Patient I001 received one course without response and subsequently underwent a successful second BMT. Patient 4321 rejected a two-antigen mismatched allogeneic marrow graft and received rhGM-CSF for graft failure while in renal and hepatic failure due to venocclusive disease (VOD). Patient 4631 received a low cell inoculum from his one-antigen mismatched marrow donor (1×10^8/kg) and rejected his graft. Patient BO99, who had received a T-cell–depleted marrow, had an increase in his ANC from 0.024×10^9/L to 0.260×10^9/L with the first course, but no response to the second course of rhGM-CSF. Patient 4588 died of progressive infection 9 days after starting rhGM-CSF. His ANC rose from 0 to 0.220×10^9/L and his marrow cellularity increased from less than 5% to 15% after receiving eight doses of rhGM-CSF. Patient FL96 had an ANC increase from 0 to 0.350×10^9/L but was found to be in relapse with acute lymphocytic leukemia on the posttherapy marrow analysis.

Five of the nine patients who responded to rhGM-CSF had initial marrow cellularity $\geq 10\%$, whereas all nonresponders had marrow cellularity $\leq 5\%$. All of the responders, except NY97 and patient 4391, who did not have follow-up bone marrows had an increase in marrow cellularity. No signifi-

Table 3. Response of Patients Treated With rhGM-CSF for Graft Failure After Allogeneic Bone Marrow Transplantation

UPN*	Dose of GM-CSF ($\mu g/m^2/d$) × d	Day 0 of Each Treatment	Marrow Genotype†		ANC × 10^9/L			Fever ≥ 38°C		Grade GVHD		Percent Bone Marrow Cellularity		Graft Status on Survival Day		Survival (d)	Relapse (d)
			Pre GM-CSF	Post GM-CSF	Pre GM-CSF	Last Day	2 wks Post GM-CSF	Pre GM-CSF	Last Day	Pre GM-CSF	Last Day	Pre GM-CSF	Last Day	ANC > 0.5 × 10^9/L	Platelet Transfusions/wk		
I001	60 × 14	93	—	H	.230	.240	.260‡	+	−	0	0	<5	<5	(+)	0	576+	
4421	60 × 21	28	—	H	0	0		+	−	0	0	10	60				
	120 × 14	78	H	H	.290	.730	2.07	−	−	0	0	10	130	(+)	0	395+	
4402	60 × 14	37	D	D	.340	3.39	3.76	+	−	II	I	<5	15	(+)	0	434+	
B099	60 × 21	138	—	—	.024	.260		+	−	0	0	<5	20				
	120 × 14	171	—	—	0	0		+	+	0	0	10	10	(−)	5	192	
4588	120 × 8	26	—	—	0	.220		+	+	0	0	<5	15§	(−)	5	35	
4321	240 × 13	34	H	H	0	0		+	+	0	0	<5	<5	(−)	6	48	
4602	250 × 14	30	—	—	.360	5.82	5.00	+	−	I	I	15	25	(+)	3	56	
4628	250 × 8	21	—	—	.090	10.94	15.57	+	−	I	I	25	30	(+)	0	240+	
4631	250 × 14	28	—	H	0	.010	0	−	−	II	I	<5	<5	(−)	5	90	
4594	250 × 7	79	D	D	.120	11.97	6.90	+	−	II	I	25	50	(+)	0	299+	
4599	250 × 14	102	D	D	.260	1.27	1.10	+	−	I	I	10	100	(+)	2	138	
NY97	250 × 14	90	H	H	0	.030		+	−	0	0	<5	<5				
	250 × 14	111	H	H	.280	.930	1.80	−	−	0	0	<5	<5	(+)	0	273+	
FL96	250 × 14	38	—	—	0	.350	.400	+	−	0	0	<5	rel	(−)	4	90	55
OS95	250 × 14	102	D/H	D/H	.060	.528	.304	−	−	0	0	<5	10	(+)	0	330+	
4391	250 × 14	114	D	—	.270	1.30		+	−	II	II	<5	−	(+)	2	135	

*UPN, unique patient number.

†Marrow genotype: H, host; D, donor. Assessed by x-y in situ hybridization studies or RFLP analysis.

‡After preconditioning with cyclophosphamide and ATG, patient I001 received a second bone marrow transplant and now has stable engraftment (ANC > 1,000/mm^3).

§Bone marrow done on day 7 after starting GM-CSF.

cant change in marrow cellularity was observed in the nonresponders when marrow analyses were performed (see Table 3). Seven of the 15 patients (UPNs 4628, OS95, NY97, 4594, 4421, 4402, and I001) were alive 75 days after initiation of rhGM-CSF and all had an ANC $\geq 0.5 \times 10^9$/L. There was no evidence for stimulation of platelet or red blood cell production concurrently with granulocytes by rhGM-CSF.

Response in patients with graft failure after autologous or syngeneic BMT. Eleven of the 21 autologous and 1 syngeneic BMT patient met the criteria for response. The mean ANC of the entire group of autologous or syngeneic BMT patients increased from $0.104 \pm 0.130 \times 10^9$ (mean ± SD) per liter (0 to 0.472×10^9/L) at start of treatment to $0.964 \pm 1.010 \times 10^9$/L (0 to 4.190×10^9/L) on the last day of the final course of rhGM-CSF ($P = .00047$; paired t-test). Nine of the 12 patients who reached an ANC of 0.500×10^9/L did so with their first course. Two patients required a second course and one responded after three courses (see Table 4). The initial increase in ANC was observed between days 5 and 10 after initiation of rhGM-CSF in 11 of the responding patients, while one patient (UPN 4050) had an ANC increase within 48 hours.

Ten patients did not respond to rhGM-CSF. Five of these nonresponders also failed second courses (3958, 4325, SJ83, SK80, and UT84). Two of these (3958 and 4325) have died of pancytopenic complications and two (SJ83 and UT84) have died of relapse (see Table 4). One (4040) failed a third course and is still alive but remains platelet transfusion-dependent with an ANC of 0.6×10^9/L. Six survived long enough after discontinuation of rhGM-CSF therapy to spontaneously achieve ANCs $\geq 0.5 \times 10^9$ per liter (HO90, SC87, OM94, SK80, UT84, and 4040) and one died without a response after receiving only four doses of rhGM-CSF (UC85).

Twenty of 22 patients had marrow cellularities of ≤5% just before receiving rhGM-CSF. The two patients with marrow cellularities of 10% and 20% responded to rhGM-CSF (see Table 4). Seven of 10 responders had an increase in marrow cellularity to ≥10%. One of nine responders (UT84)

Table 4. Response of Patients Treated With rhGM-CSF for Graft Failure After Autologous or Syngeneic Bone Marrow Transplantation

UPN*	Dose of GM-CSF ($\mu g/m^2/d$) × d	Day 0 of Each Treatment	ANC × 10^9/L			Fever ≥38°C		Percent Bone Marrow Cellularity		Graft Status on Survival Day		Survival (d)	Relapse (d)
			Pre GM-CSF	Last Day	2 wks Post GM-CSF	Pre GM-CSF	Last Day	Pre GM-CSF	Last Day	ANC > 0.5×10^9 L	Platelet Transfusions/wk		
BO98	60 × 14	45	.036	.384		+	+	<5	<5				
	120 × 21	60	.252	.500	.594	+	–	<5	10	(+)	0	481+	
4319	120 × 14	31	.010	4.190	1.100	+	–	<5	35	(+)	0	396+	
3958	120 × 14	29	.020	.080		+	–	<5	<5				
	240 × 14	58	.010	.220	.210	+	+	5	5				
	500 × 7/1,000 × 7	52	0	.130	0	–	–	<5	5	(–)	6	85	
OM99	120 × 14	41	0	.336		–	–	<5	<5				
	250 × 14	68	.172	.476		–	–	<5	<5				
	500 × 14	95	.472	1.701	.750	–	–	<5	15	(+)	1	267	
SF96	240 × 14	42	.150	1.300	1.150	+	–	<5	10	(+)	0	321+	246
4050	240 × 14	70	.250	2.070	2.500	+	–	10	20	(+)	0	608+	
4040	240 × 14	39	0	0		+	–	<5	<5				
	500 × 14	53	0	0	0	–	–	<5	<5				
	1,000 × 14	74	0	.50	.50	–	–	<5	<5	(+)	4	366+	
4325	500 × 14	30	0	.200		–	–	<5	<5				
	500 × 7/1,000 × 7	52	0	.130	0	–	–	<5	5	(–)	6	81	
4570	250 × 14	26	.010	.990	1.000	+	–	<5	–	(+)	0	323+	
4501	250 × 14	32	.070	1.430	1.010	–	–	20	50	(+)	1	320+	
OM91	250 × 14	33	0	0	—	+	–	<5	<5				
	250 × 14	66	.280	1.020	1.036	+	–	<5	–	(+)	3	134	134
HO90	250 × 14	70	.216	.300	.460	+	+	<5	<5	(+)	4	153	
SC87	250 × 14	37	.030	.030	.270	+	+	<5	<5	(+)	0	260+	
GT86	250 × 8	21	0	1.636	2.403	+	–	<5	<5	(+)	4	66	
UC85	250 × 4	24	0	0	—	+	+	<5	–	(–)	7	28	
UT84	250 × 14	26	.080	.320		+	+	<5	10				
	250 × 14	54	.135	.120	.020	+	+	5	<5	(–)	5	193	120
SJ83	250 × 14	56	0	0	0	–	–	<5	<5				
	250 × 5	77	0	Rel	—	–	–	<5	Rel	(–)	7	94	82
OM94	250 × 14	31	0	0	.028	+	–	<5	<5	(+)	0	257+	105
MI79	250 × 14	35	.090	1.804	3.00	+	–	<5	–	(+)	0	230+	
4721	250 × 14	31	.160	1.790	—	+	–	<5	60	(+)	2	54	54
OM81	250 × 14	36	.115	1.112	1.500	–	–	<5	<5	(+)	0	582+	
SK80	250 × 14	35	.100	.150		–	–	<5	<5				
	500 × 14	57	.200	.450	.700	–	–	5	5	(+)	0	565+	

*UPN, unique patient number.

had a transient increase in marrow cellularity after one course of rhGM-CSF therapy; however, after a second course of rhGM-CSF his marrow cellularity decreased to less than 5%. Follow-up marrow samples were not obtained in three patients. Sixteen of the patients were alive 75 days after initiation of rhGM-CSF and 14 had an ANC $\geq 0.5 \times 10^9$/L. No increase in platelet counts was noted during rhGM-CSF therapy, and rhGM-CSF did not stimulate a reticulocytosis in any patient.

Effect on infection. Fevers (temperature greater than 38°C) were present in 13 of 15 allogeneic BMT patients before treatment with rhGM-CSF. Five patients had bacteremia or fungemia (*Escherichia coli,* UPN I001; *Candida albicans,* UPNs 4588, 4321, 4602; *C tropicalis,* UPN 4628); two had viral infections (adenovirus, UPN OS95; adenovirus, CMV, resistant herpes simplex, UPN 4391); and one had liver, spleen, and brain abscesses (UPN 4602). Fevers resolved in all responders (see Table 3). Fungemia present before GM-CSF was started cleared in patients UPN 4628 and 4602; nonetheless, the latter died of a cerebral hemorrhage, and *C albicans* was detected in the brain, liver, and spleen at autopsy. Another responder (UPN 4391) died of progressive viral infection. Four of the six nonresponders died of infection, two of aspergillus pneumonia (UPNs B099 and 4321), and two of septicemia (UPNs 4631 and 4588).

Fever was present in 16 of 22 autologous and syngeneic BMT patients before treatment with rhGM-CSF. Five of the 22 patients had bacteremia or fungemia, (pseudomonas, 4319; *E coli,* SJ83; *C albicans,* 4570; fusarium, UT84; coagulase negative staphylococcus, OM91). Three patients had pneumonia (legionella, UC85; unknown, GT86; HO90) and one patient had a cellulitis (UPN 4040). Fevers and bacteremia or fungemia resolved in all responders (see Table 4). During rhGM-CSF therapy, fevers did not resolve in 5 of 10 nonresponders. Pneumonia progressed in patient UC85 and resolved in patients GT86 and HO90. Cellulitis resolved in patient 4040.

Effects on engraftment and graft-versus-host disease (GVHD). Of three patients with graft rejection (only host cells in circulation), two responded to rhGM-CSF with recovery of host hematopoiesis. In the third patient, no response was noted (see Table 3). In four patients, only donor hematopoietic cells were detected at the time of treatment. All four responded to rhGM-CSF. In one patient (OS95), five of seven metaphases from stimulated peripheral blood lymphocytes were of donor origin before starting rhGM-CSF. After rhGM-CSF therapy, 5 of 10 circulating cells were of donor origin. As shown in Table 3, seven patients had evidence of grade I or II GVHD just before starting rhGM-CSF. None of the patients had an exacerbation of GVHD during rhGM-CSF therapy.

Effect of marrow purging. None of the seven patients who received autologous marrow treated in vitro with either 4-hydroperoxycyclophosphamide (4-HC) or etoposide (see Tables 2 and 4) responded to rhGM-CSF, while 12 of the 15 patients who received unpurged autologous or syngeneic marrows (n = 9) or monoclonal antibody purged marrows (n = 6) responded ($P < .0007$, Fisher exact test). Only 2 of 7 patients (UPNs 4040 and SK80) who developed graft failure after grafting with chemically purged marrow survived (see Table 4).

Toxicity. The four autologous BMT recipients who were administered doses of rhGM-CSF $\geq$ 500 $\mu g/m^2/d$ (six courses) developed myalgias and bone pain during the infusion. Low doses of demerol were administered for relief. Myalgias and bone pain resolved regardless of whether demerol was administered within 2 hours after completion of the rhGM-CSF infusion. One of these (UPN OM99), also developed an acute, aseptic, transudative pericardial effusion requiring external drainage after her third course of rhGM-CSF. She also developed hydrocephalus 2 weeks after discontinuation of rhGM-CSF. The etiology of the pericardial effusion and hydrocephalus are unknown, but given the lack of other causes, rhGM-CSF was assumed to be the cause.

At doses of ≤ 250 $\mu g/m^2/d$ (18 courses in allogeneic BMT patients had 28 courses in autologous or syngeneic BMT patients), toxicity ascribed to rhGM-CSF was observed in one patient (SC87) who developed sternal and joint pain requiring narcotic analgesics. rhGM-CSF was discontinued in patient GT86 after eight doses because an ANC $\geq 0.5 \times 10^9$/L had already been achieved and other medical therapy had induced renal failure. Bilirubin increased in three patients (UPNs 3958, 4588, and GT86) and diminished in two others (UPNs 4319 and 4321). No significant effects on creatinine, albumin, weight, or other organ systems were detected. Seven of nine allogeneic BMT patients and 11 of 12 autologous BMT patients who responded to rhGM-CSF had significant improvement in performance status during therapy and were discharged from the hospital. There was no immediate improvement in performance status during therapy among the 16 patients who did not respond.

Survival. Of the 37 patients treated with rhGM-CSF, 19 are alive (median follow-up 330 (range 240 to 608 days post BMT). The actuarial survival of the 37 patients 100 days and 1 year after the day they received rhGM-CSF was 59% (95% confidence interval [CI] of 44% to 75%) and 50% (95% CI of 36% to 60%), respectively.

Three of nine allogeneic BMT patients who responded to rhGM-CSF died. UPN 4391 died of systemic viral infection, UPN 4602 had a cerebral vascular hemorrhage, and UPN 4599 died of CMV pneumonia. Death occurred 7, 12, and 22 days, respectively, after discontinuation of rhGM-CSF. The other six responders are alive without infection and are disease-free 240 to 434 days post BMT. Five of the six nonresponders have died, four of infection and one of recurrent disease. The one nonresponder (I001) who survived underwent a successful second allogeneic BMT (see Table 3).

Four of 12 responders after autologous BMT have died. Patients 4721 and OM91 died of their primary disease, patient GT86 died of a cerebral hemorrhage, and patient OM99 died suddenly of unknown causes. Deaths occurred 9, 54, 29, and 158 days after discontinuation of rhGM-CSF, respectively. The other eight patients are alive without infection and seven are disease-free 230 to 608 days post BMT. Six of 10 nonresponders died; two of pneumonia (aspergillus and mycobacterium, 3958; legionella, UC85),

one of septicemia (4325), one of a perforated gastric ulcer (HO90), and two of relapse (SJ83, UT84). All of the surviving nonresponders eventually achieved an ANC $\geq 0.5 \times 10^9$/L after discontinuation of rhGM-CSF (OM94, SC87, SK80, and 4040). However, patient SC87 relapsed of primary disease while patients OM94, SK80, and 4040 remain disease-free (see Table 4).

Retrospective control group. One hundred fifty-five patients transplanted in Seattle, WA, between January 5, 1981 and May 27, 1987 who fulfilled the criteria for graft failure as defined in this study were identified. In Table 5, salient characteristics of these patients are compared with those of the patients treated with rhGM-CSF. However, a larger percentage of the retrospective control group received allogeneic transplants, probably reflecting a recent increased usage of autologous marrow. The actuarial survival of the 155 retrospective control patients with graft failure was 32% at day 100 and 23% (95% CI, 16% to 29%) at 1 year posttransplant. This compares to 75% actuarial survival at day 100 and 47% (95% CI, 38% to 56%) at 1 year in the 1,524 patients without graft failure from January 5, 1981 to May 27, 1987 ($P = .0001$, see Fig 1). Survival was not significantly different ($P = .32$, Wilcoxon) when comparing autologous BMT patients to allogeneic BMT patients with marrow graft failure. Infection was the leading cause of death in the historical control patients accounting for 59% of deaths. The overall mortality rate was 45%.

Table 5. Characteristics of Retrospective Control Patients With Graft Failure Compared With Prospective Patients With Graft Failure Who Received rhGM-CSF

Characteristics	Control Patients (n* = 155)	rhGM-CSF–Treated Patients (n* = 37)
Mean age (range)	26 (2-57)	26 (3-65)
Sex: M/F (%)	61/39	59/41
Type of BMT (%):		
Matched allogeneic	53	27
Partially matched allogeneic	30	5
Unrelated allogeneic	2	8
Autologous	14	57
Syngeneic	1	3
Disease (%):		
ANL†	34	27
CML†	25	11
ALL†	21	16
NHL/HD†	8	31
AA†	3	5
MD†	4	0
ID†	3	0
ST†	0	10
Preparative regimen (%):		
Containing TBI‡	92	54
Not containing TBI‡	8	46

*n, number.

†ANL, acuted nonlymphocytic leukemia; CML, chronic myelogenous leukemia; ALL, acute lymphocytic leukemia; NHL, non-Hodgkin's lymphoma; AA, aplastic anemia; MN, malignant neoplasm; MD, myelodysplastic syndrome; ID, immunodeficiency syndrome; ST, solid tumor.

‡TBI, total body irradiation.

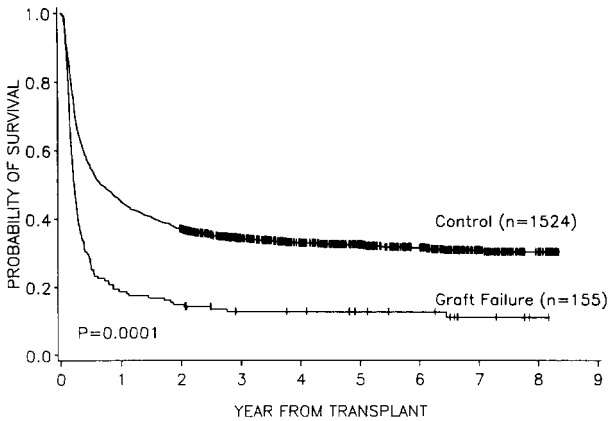

Fig 1. From 1981 to 1987, 155 consecutive patients who fulfilled the study definition of graft failure were identified. Their disease-free survival (Kaplan-Meier) is shown compared with 1,524 patients over the same time period who did not develop graft failure; $P < .0001$, Wilcoxon.

In comparison, infection was the primary cause of death in 21% of the rhGM-CSF–treated patients with graft failure. The actuarial risk of relapse for patients with graft failure was 16% at day 100 and 43% at 1 year. The actuarial risk of relapse among the 35 patients transplanted for malignancy who were administered rhGM-CSF for graft failure was 10% ± 5% at day 100. Seventy-five days after eligibility for rhGM-CSF, only 39% of the control patients were alive. Most deaths were related to pancytopenic complications. Of the patients who survived 75 days after eligibility, 82% achieved stable ANCs above 0.5×10^9/L. Sixty-two percent of the rhGM-CSF–treated patients were alive 75 days after starting the initial course of rhGM-CSF. Ninety-one percent of the surviving rhGM-CSF–treated patients had ANCs above 0.5×10^9/L at day 75. This was not different than the recovery in the surviving historical patients. A direct comparison of overall survival between the 155 historical control patients and the 37 patients who received rhGM-CSF is shown in Fig 2. The GM-CSF–treated patients had a significantly better survival than did the historical controls ($P = .001$, Wilcoxon).

DISCUSSION

Given the high incidence of serious or fatal infections in patients with granulocytopenia due to marrow graft failure, we insitituted the present study to evaluate the safety and efficacy of rhGM-CSF. Patients with low performance status were not excluded. Only patients with graft failure due to leukemic relapse and patients with transient myelosuppression thought to be drug-induced were not offered rhGM-CSF. The results demonstrate that the majority of patients who receive rhGM-CSF respond by increasing their ANC to above 0.5×10^9/L within 2 weeks of therapy, a level associated with a reduced risk of bacterial infection. This effect is likely to be due to the proliferative and differentiative effects of rhGM-CSF on committed progenitor cells. rhGM-CSF also enhances the functional capacity of myeloid cells and thus may decrease the risk of death from infection at low granulocyte levels, allowing the patient to survive until

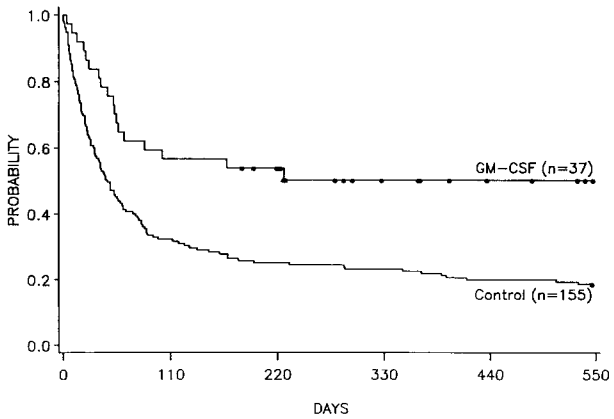

Fig 2. Comparison of survival (Kaplan-Meier) between 155 consecutive historical patients with graft failure and 37 graft failure patients who received rhGM-CSF. Day 0 in the control patients is the day the definition of graft failure was fulfilled, and the day 0 of the study patients was the day of initiation of rhGM-CSF.

spontaneous marrow recovery occurs. It is not possible from the present data to be sure that all increases in ANC were due to rhGM-CSF rather than spontaneous recovery. However, compared to historical control patients with graft failure after either autografts or allografts, rhGM-CSF–treated patients appeared to have an increased survival rate. The only significant toxicity observed with rhGM-CSF occurred at a dose of 500 $\mu g/m^2/d$; when administered at a dose of 250 $\mu g/m^2/d$, it was well-tolerated.

GM-CSF can stimulate myeloid leukemic blast cell proliferation in vitro and possibly in vivo, which raises the concern that treatment of patients transplanted for myeloid leukemia might lead to early leukemic relapse.[22,23] In the 14 patients who had nonlymphocytic leukemia before BMT and who were treated with rhGM-CSF, there was only one relapse. While these data do not exclude the possibility of blast cell stimulation, no evidence for such a phenomenon was seen in this study.

Another concern in using rhGM-CSF after allografting is its potential to exacerbate GVHD. GM-CSF can stimulate macrophage production of interleukin-1 (IL-1),[24] which can stimulate macrophage-mediated T-cell activation.[25] Theoretically, enhanced lymphocyte activity could exacerbate GVHD. However, in the present trial, of the five allogeneic patients with either only donor cells or with mixed chimerism, no exacerbation of GVHD was seen.

Treatment of autologous marrows with 4-HC or etoposide has been used to selectively remove leukemic progenitor cells before reinfusion.[12] A potentially important observation in this study was the lack of response to rhGM-CSF in the seven recipients of chemically purged marrow grafts. This differs significantly from the remaining autologous patients who either received unpurged or monoclonal antibody-purged marrow grafts. The lack of a response to rhGM-CSF in patients who received chemically purged marrow may be due to damage to progenitor cells or due to the underlying disease process. Six of the seven patients with chemically purged marrows had acute nonlymphocytic leukemia (ANL), while none of the other 15 patients had ANL. Damage to stem cells due to chemical agents as a mechanism for lack of response to rhGM-CSF is also suggested by data from another trial, where rhGM-CSF was administered to patients with acute lymphoblastic leukemia who received chemically purged autografts. No significant response to rhGM-CSF was observed.[26] It is still possible that an increase in the functional capacity of neutrophils can be achieved with rhGM-CSF sufficient to benefit patients who receive chemically purged marrows. Patients who receive chemically purged marrow might respond to rhGM-CSF at higher doses, with a prolonged course, or a later time period after BMT.

In summary, treatment with rhGM-CSF was nontoxic and associated with increases in neutrophil production in a substantial proportion of patients with marrow graft failure. Responses to rhGM-CSF were associated with resolution of fevers and infection, and improved survival when compared with historical control patients. In view of the poor prognosis of patients with graft failure after BMT and the lack of alternative effective therapy, the present data suggest that patients with marrow graft failure should receive a trial of rhGM-CSF.

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