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Optimization of recombinant human erythropoietin therapy after allogeneic hematopoietic stem cell transplantation

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Objective. **Allogeneic hematopoietic stem cell transplantation (HSCT) is associated with prolonged anemia caused by defective erythropoietin (Epo) production. We enrolled 34 recipients of an allogeneic HSCT in three consecutive trials to determine the optimal utilization of recombinant human erythropoietin (rhEpo) therapy in this setting.**

Materials and Methods. **In the first trial (n = 7), rhEpo 1400 U/kg/week was given from day 1 until a hemoglobin (Hb) level of 10 g/dL was achieved, for a maximum of 60 days. In the second trial, rhEpo 500 U/kg/week was given to achieve Hb levels of 13 to 14 g/dL in 13 anemic patients with fatigue 56 to 1440 days after transplant. In the third trial, rhEpo was scheduled to start on day 35 in 14 patients at a dose of 500 U/kg/week with the aim of achieving Hb levels of 13 to 14 g/dL.**

Results. **In trial 1, erythroid recovery to 1% reticulocytes and red blood cell transfusion independence were faster, but the number of transfusions was not reduced compared to 10 controls. Responses were brisk in trial 2, with transfusion independence achieved after a median of 1 week in 12 of 13 patients, and 2-g Hb increments or Hb values of 11, 12, and 13 g/dL after 6, 7, 10, and 10 weeks, respectively. Transfusions were significantly reduced in the first month of rhEpo therapy. In trial 3, transfusion independence was obtained after a median of 1 week in 13 of 14 patients, and 2-g Hb increments or Hb values of 11, 12, and 13 g/dL after 3, 4, 6, and 8 weeks, respectively. Transfusions rates were considerably reduced compared to the previous month in the same patients or compared to controls undergoing peripheral blood or marrow transplant without rhEpo.**

Conclusions. **Anemia after allogeneic HSCT is exquisitely sensitive to rhEpo. The benefit is minimal when it is given early post-transplant, as used in all trials to date. However, the rate of major response is greater than 90% when rhEpo is started after day 35. These data provide the basis on which to conduct a prospective, randomized, placebo-controlled trial of rhEpo therapy after allogeneic HSCT.**

Erythropoietin (Epo) is the critical regulatory factor of erythropoiesis, and recombinant human erythropoietin (rhEpo) has become a well-established treatment for chronic renal failure patients. In these subjects, endogenous serum Epo levels are very low [1], and administration of rhEpo to restore adequate levels of the hormone permits correction of the anemia [2].

In patients with normal kidney function, serum Epo levels increase exponentially when an anemia develops [1]. After high-dose chemotherapy, serum Epo levels first rapidly increase to disproportionately high levels for 1 to 3 weeks, with peak values usually observed in the first week after the conditioning regimen [3–14]. However, after allogeneic hematopoietic stem cell transplantation (HSCT), the Epo response to anemia then generally becomes impaired, resulting in inappropriately low levels of Epo for the degree of anemia and prolonged anemia [7–9,13–15]. This is specific for allogeneic transplant because serum Epo levels remain adequate throughout the post-transplant course in recipients of autologous marrow or peripheral blood stem cell transplant (PBSC) [6,7,10–15].

It is not surprising that rhEpo therapy has consistently failed to accelerate erythropoietic recovery or to decrease

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transfusion requirements after autologous HSCT [16–22]. In contrast, clinical trials of rhEpo therapy after allogeneic bone marrow transplantation (BMT) resulted in accelerated erythroid engraftment and some reduction in transfusion requirements [17,18,23–30]. However, in all of these studies, the rhEpo dose used was very high (greater than 1000 U/kg/week) and thus the cost was prohibitive. In addition, rhEpo was only given from day 1 through days 30 to 55 or until erythroid engraftment, a period when patients often are afflicted by serious toxic, infectious, or immunologic complications. This may explain why placebo-controlled trials have shown little clinical benefit, including little reduction in transfusion requirements, other than biologic signs of accelerated erythropoiesis [18,27,28].

We report here our experience with three consecutive trials of rhEpo therapy after allogeneic HSCT. In the first trial, we administered Epo in the early post-transplant period but failed to achieve significant clinical benefit. We then used rhEpo in a manner more consistent with the pathophysiology of erythropoiesis after allogeneic transplantation [14]. In the second trial, rhEpo was given to patients with persisting anemia late after transplant, and excellent responses were obtained with complete correction of hemoglobin (Hb) values in the vast majority of the patients. In the third trial, we examined whether starting rhEpo around day 35, when the graft was well established and just before endogenous Epo deficiency became prominent [14], would produce optimal responses. This approach proved very successful, and responses were brisk and sustained. This study should form the basis for a more rational use of rhEpo after allogeneic HSCT and for the design of Phase III studies investigating such clinical endpoints as transfusion requirements, iron overload, and organ damage, as well as quality of life.

Patients and methods

Patients

Thirty-four patients (22 males and 12 females; age 8 to 58 years, median 43) were enrolled. Marrow (n = 8) or PBSC (n = 26) transplants were collected in HLA-identical siblings (n = 19) or alternative donors (n = 15). The diagnoses were acute myeloid leukemia (AML; n = 14), chronic myeloid leukemia (CML; n = 7), acute lymphoid leukemia (ALL; n = 5), non-Hodgkin's lymphoma (NHL; n = 4), myelodysplastic syndrome (MDS; n = 1), severe aplastic anemia (SAA; n = 2) and essential thrombocythemia (n = 1). Their clinical characteristics are summarized in Table 1. Conditioning before transplantation consisted of various combinations of high-dose chemotherapy with (n = 27) or without (n = 7) total body irradiation. Graft-vs-host disease (GVHD) prophylaxis was carried out with cyclosporine with (n = 15) or without (n = 19) short methotrexate. All patients were taking cyclosporine when rhEpo was started. Single-donor platelet transfusions were given if platelet counts decreased below 15×10^9/L. The trigger for packed red blood cell (RBC) transfusions decreased in recent years at our institution so that patients in the first trial were transfused when the hemoglobin level decreased below 9 g/dL while those in the second and third trials received RBC transfusions when the Hb was below 8 g/dL. Patients included in the first trial were compared to 10 patients (3 MDS, 2 CML, 2 NHL, 1 AML, 1 SAA, and 1 neuroblastoma) undergoing an allogeneic PBSC transplant (9 HLA-identical and 1 one-mismatch sibling) in the same period but not receiving rhEpo (control group). Patients in the third trial were retrospectively compared to an historic group of 47 BMT patients previously reported [14] as well as to 7 patients (2 MDS, 2 CML, 1 NHL, 1 AML, 1 SAA) of the PBSC control group with a follow-up of at least 5 months.

RHuEpo treatment

In the first trial (n = 7), rhEpo 1400 U/kg/week was given intravenously (IV) on a daily basis from day 1 after peripheral blood HSCT until an unsupported Hb level of 10 g/dL was achieved, for a maximum of 60 days. The rhEpo (Eprex) was kindly provided by Janssen-Cilag (Beerse, Belgium).

In the second trial (n = 13), rhEpo was given subcutaneously (SC) three times per week to achieve Hb levels of 13 g/dL in patients who complained of fatigue and showed persisting anemia 56 to 1440 days after bone marrow or peripheral blood HSCT. The dose was 500 U/kg/week, but three patients (no. 9, 10, and 17) with anemia of renal failure received only one third to one half of that dose and the dose was increased to 700 to 1000 U/kg/week in five slowly responding patients. The rhEpo was kindly supplied by either Janssen-Cilag or Roche (Neorecormon, Roche, Brussels, Belgium).

In the third trial (n = 14), rhEpo was scheduled to start on day 35 after bone marrow or peripheral blood HSCT at a dose of 500 U/kg/week (given SC in two divided doses) with the aim of achieving and maintaining Hb levels of 13 g/dL. If there was an active complication such as infection or GVHD on day 35, the introduction of rhEpo was delayed until the complication resolved. If the Hb had not increased by at least 1 g/dL after 1 month, the dose was doubled (two patients). If no response was achieved after a total of 80 days of therapy, rhEpo was discontinued (one patient). The rhEpo (Neorecormon) was kindly provided by Roche.

In the second and third trials, once the target Hb (13 g/dL) was achieved, the dose of rhEpo was reduced so as to use the lowest dose capable of maintaining Hb between 12 and 14 g/dL. No dose reduction was necessary in the first trial. No iron supplements were given.

Laboratory analyses

Complete blood counts were determined in a Technicon H2 cell counter (Bayer, Tarrytown, NJ, USA). Percentages of reticulocytes were obtained by an automated cytofluorometric method [31]. Serum Epo levels were measured by a commercially available radioimmunoassay (Incstar Corp., Stillwater, MN, USA). Based on regression equations obtained in appropriate reference subjects between hematocrit (Hct) on the one hand and log (Epo) on the other, predicted log (Epo) values were derived for each Hct, and O/P ratios of observed/predicted Epo values were calculated [32]. Serum soluble transferrin receptor (sTfR), a quantitative measure of total erythropoietic activity, was measured by a commercially available ELISA (R&D, Minneapolis, MN, USA). Normal values range from 3000 to 7000 μg/L. Serum iron, transferrin saturation, and ferritin were measured by standard methods.

Evaluation of response to rhEpo

A major response (responder) was defined by an Hb increment greater than 2 g/dL and achievement of an Hb level greater than 10

Table 1. Characteristics of the patients in the three trials

Patient no.	Diagnosis	Age (years)	Sex	Source of stem cells	Type of donor	ABO Rhesus (Recipient/donor)	Acute GVHD	Chronic GVHD
Trial 1								
1	AML	47	M	PBSC	Sibling, HLA_{id}	A+/A+	III	NA
2	AML	8	M	PBSC	Father, 1 mismatch	A+/A−	III	NA
3	NHL	58	F	PBSC	Son, HLA_{id}	A+/A+	III	NA
4	CML	43	F	PBSC	Sibling, HLA_{id}	A+/A+	III	NA
5	AML	16	M	PBSC	Mother, HLA_{id}	A+/O+	IV	NA
6	AML	31	M	PBSC	Sibling, HLA_{id}	O+/O+	II	NA
7	AA	47	M	PBSC	Sibling, HLA_{id}	A+/A+	I	NA
Trial 2								
8	AML	54	M	BM	Sibling, HLA_{id}	A+/A,+	NA	Extensive
9	CML	48	M	BM	Unrelated, HLA_{id}	O−/O+	NA	Extensive
10	AML	24	M	BM	Unrelated, HLA_{id}	O+/A+	NA	Extensive
11	CML	46	M	BM	Unrelated, HLA_{id}	O+/O+	NA	Extensive
12	AML	8	M	BM	Sibling, HLA_{id}	O+/O+	NA	No
13	AML	18	M	PBSC	Sibling, HLA_{id}	O+/O−	NA	No
14	ALL	15	F	PBSC	Sibling, HLA_{id}	AB+/B+	II	Extensive
15	AML	40	M	PBSC	Sibling, HLA_{id}	O+/O+	NA	No
16	ALL	17	M	BM	Unrelated, HLA_{id}	O−/O+	IV	NA
17	CML	54	M	PBSC	Sibling, 1 mismatch	A+/O+	NA	Limited
18	AML	48	F	PBSC	Daughter, 1 mismatch	A+/O+	NA	Limited
19	CML	49	M	PBSC	Sibling, HLA_{id}	O+/A+	NA	No
20	AML	56	F	PBSC	Sibling, HLA_{id}	O+/A−	0	No
Trial 3								
21	AML	27	M	PBSC	Sibling, HLA_{id}	O−/A+	0	NA
22	MDS	51	M	PBSC	Sibling, HLA_{id}	O+/A+	0	No
23	ALL	27	M	PBSC	Mother, 1 mismatch	O+/O+	0	Extensive
24	ALL	23	M	PBSC	Sibling, HLA_{id}	A+/A+	0	No
25	ALL	18	M	PBSC	Sibling, HLA_{id}	A+/O+	0	Extensive
26	NHL	48	F	PBSC	Sibling, 1 mismatch	A+/A+	0	Extensive
27	NHL	44	F	PBSC	Sibling, 1 mismatch	A+/A+	IV	No
28	CML	26	M	BM	Unrelated, 1 mismatch	A+/O+	0	NA
29	ET	31	F	BM	Unrelated, HLA_{id}	A+/A+	0	No
30	NHL	50	M	PBSC	Sibling, HLA_{id}	B+/O+	II	Extensive
31	CML	47	F	PBSC	Daughter, 1 mismatch	A+/A−	II	Extensive
32	AML	51	F	PBSC	Sibling, HLA_{id}	A−/A−	I	NA
33	AML	49	F	PBSC	Sibling, HLA_{id}	A+/A+	0	No
34	AA	44	F	PBSC	Sibling, HLA_{id}	A+/A+	0	No

AA = aplastic anemia; ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; BM = bone marrow; CML = chronic myeloid leukemia; ET = essential thrombocythemia; F = female; GVHD = graft-vs-host disease; HLA_{id} = HLA identical; M = male; MDS = myelodysplastic syndrome; NA = not applicable because no rHuEpo was given before day 100 (aGVHD) or after day 100 (cGVHD); NHL = non-Hodgkin's lymphoma; PBSC = peripheral blood stem cells.

g/dL without transfusion needs. The response was considered minor if only one of these criteria was fulfilled. In the second and third trials, the response was considered as complete when Hb reached the target value of 13 g/dL.

Statistical methods

Unpaired and paired Student's *t*-tests were used to compare biologic variables in two groups or to compare baseline value with later measurements in the same group of patients, respectively. Welsh's correction was used in case of unequal variance. Number of transfusions in the same or in different groups of patients were compared using Wilcoxon matched pair or Mann-Whitney *U*-tests, respectively. R correlation coefficients between two variables were computed in least squares regression equations. Times to response to rhEpo therapy as well as times to hematopoietic recovery were studied by life-table analyses, and Wilcoxon rank tests were used for comparisons between groups. Statistical analyses were carried out with Graphpad Prism (Graphpad Software, San Diego, CA, USA).

Results

First trial

Compared to the control group, erythroid recovery was significantly faster in the study group. Median time to 1% reticulocytes was 12 days (range 10–25) in the study group vs 27 days (range 13–72) in the control group ($p = 0.0177$). Neutrophil and platelet engraftments were not different. Five patients in the study group reached RBC transfusion

Table 2. Response to rhEpo treatment in the first trial

No.	rhEpo duration (days)	Reticulocytes ($\times 10^9$/L)			Hb (g/dL)			No. RBC transfusions (days 1–60)	Comments
		Baseline	Peak	Nadir	Baseline	Peak	Nadir		
rhEpo group		Day 0	On treatment	After treatment*	Day 0	On treatment	After treatment*		
1	31†	19	39	25	9.5	Tx	Tx	17	TTP, infection
2	18	9	294	76	9.7	10.6	9.1	3	
3	60	7	341	121	9.5	11.9	Tx	12	Infection
4	60	23	211	59	10.9	10.8	9.6	15	Bleeding, VOD
5	31	24	328	NA	8.5	Tx	Tx	38	Bleeding, AAHA, GVHD Infection
6	21	13	132	46	8.7	11.3	9.1	8	
7	60	73	297	27	7.8	12.7	9.1	10	ATG
Median	31	19	294	53	9.5	11.3	9.1	12	
Control group		Day 0	Months 1–2	Months 3–5	Day 0	Months 1–2	Months 3–5		
1	0	10	249	39	8.5	13.3	Tx	10	
2	0	13	NA	NA	9.3	NA	NA	11	
3	0	3	140	58	8.1	9.6	9.7	13	
4	0	19	170	29	10.3	Tx	Tx	12	
5	0	26	ND	NA	7.8	Tx	NA	28	Infection
6	0	9	270	139	8.9	Tx	9.0	29	TTP
7	0	62	131	67	8.6	11.1	8.4	9	
8	0	8	88	NA	8.4	Tx	NA	15	VOD
9	0	10	164	NA	9.9	Tx	NA	31	GVHD, bleeding
10	0	9	138	ND	8.9	Tx	Tx	22	Infection
Median	0	10	152	58	8.7	11.1	9.0	14	

*During the 3 months after cessation of rHuEpo. †rhEpo stopped because of hypertension and heart failure.

AAHA = acute alloimmune hemolytic anemia; ATG = antithymocyte globulins; NA = not applicable; ND = not done; TTP = thrombotic thrombocytopenic purpura; Tx = transfusion; VOD = veno-occlusive disease of the liver.

independence within 30 days (Table 2). Two patients remained transfusion dependent because of hemolysis and/or bleeding. After excluding these two patients in the study group as well as one patient in the control group who also had severe bleeding, the median time to RBC transfusion independence was significantly shorter in the study group (21 days, range 7–29) than in the control group (40 days, range 21–110) ($p = 0.0007$). However, the number of RBC transfusions in the first 60 days post-transplant was not reduced: median 12 (range 3–38) in the study group vs 14 (range 9–31) in the control group (p = NS).

Second trial

Overall, responses were brisk (Table 3). Transfusion independence was achieved after a median of 1 week (range 0–23) in 12 (92%) of 13 patients. Median number of transfusions per month decreased from 2 at baseline to 0 during months 1 ($p = 0.0020$), 2 ($p = 0.0537$; $p = 0.0020$ after excluding patient 16 who bled massively after responding well to rhEpo), and 3 ($p = 0.0137$; $p = 0.0039$ after excluding patient 16) of rhEpo therapy. Median time to an Hb increment of 2 g/dL was 6 weeks, and Hb values of 11, 12, and 13 g/dL were achieved after a median of 7, 10, and 10 weeks, respectively. Complete responses were obtained in 11 (85%) of 13 patients, whose Hb increments ranged from 4.3 to 7.4 g/dL (median 6.3) and peak Hb values from 13.5 to 15.6 g/dL. Maintenance doses of 30 to 280 U/kg/week (median 135) were capable of maintaining Hb levels between 12 and 14 g/dL for periods up to 2 years. When rhEpo was discontinued, Hb decreased to a median of 9.9 g/dL within 3 months. One patient had a minor response, with transfusion independence achieved within 1 week and an Hb increment of 1.6 g/dL. However, rhEpo was stopped prematurely after 35 days because the patient developed grade IV acute GVHD with massive gastrointestinal bleeding. Finally, one patient with very poor graft function (continuing platelet transfusions and granulocyte colony-stimulating factor requirements), bleeding, and hemolysis did not respond and remained transfusion dependent.

Third trial

Responses in these patients were excellent (Table 4). Transfusion independence was achieved after 1 week in 13 (93%) of 14 patients. Median number of transfusions per month decreased from 3 at baseline to 0 in months 1 ($p = 0.0001$), 2 ($p = 0.0002$), and 3 ($p = 0.0081$) of rhEpo therapy. Hb values of 11, 12, and 13 g/dL were achieved after a median of 4, 6, and 8 weeks, respectively. Median time to an Hb increment of 2 g/dL was 3 weeks. Thus, a complete response was achieved in 12 and a major response in 1 of the 14 patients (93%). Their Hb increments ranged from 3.8 to 10.3 g/dL

Table 3. Response to rhEpo treatment in the second trial

No.	rhEpo treatment: Baseline serum Epo (mU/mL)	rhEpo treatment: Starting day after transplant	rhEpo treatment: Starting dose (U/kg/week)	rhEpo treatment: Maximal dose (U/kg/week)	rhEpo treatment: Duration (days)	Hb (g/dL): Baseline	Hb (g/dL): Peak on treatment	Hb (g/dL): Nadir after treatment*	RBC transfusions (U/month): Baseline	RBC transfusions (U/month): Month 1	RBC transfusions (U/month): Month 2	RBC transfusions (U/month): Month 3	Comments
8	55.5	1444	500	700	88†	7.1	14.5	10.0	2	0	0	0	Infection, bleeding, CRF, cGVHD
9	37.2	224	150	150	400	7.8	13.7	9.8	4	1	0	0	CRF
10	75.7	182	280	830	700	8.9	13.5	ND	6	3	2	6	PMF, CRF, cGVHD, infection
11	143.6	301	500	500	196	8.7	15.2	10.1	0	0	0	0	Infection
12	11.1	189	500	500	42†	8.0	14.3	9.1	1	0	0	0	
13	40.9	105	500	870	215	8.6	15.6	11.6	4	0	0	0	PMF, infection
14	37.7	84	500	500	200	7.5	14.1	8.0	2	1	0	0	Pancreatitis, infection, surgery
15	38.1	126	500	500	140	8.9	15.2	9.9	0	0	0	0	
16	97.1	56	500	500	35‡	8.0	9.6	Tx	4	0	18‡	6‡	aGVHD, bleeding, infection
17	ND	490	180	180	42†	9.2	13.5	10.6	0	0	0	0	
18	38.4	154	500	1000	65	8.3	13.6	12.2	2	2	1	0	PRCA, infection
19	95.9	126	500	800	360+	7.4	13.7	—	4	1	2	0	
20	ND	63	500	1000	80‡	7.6	Tx	Tx	14	4	4	4	Dialysis, bleeding, TTP, infection, PMF
Median	48.2	154	500	500	—	8.0	13.7	9.9	2	0	0	0	

*During the 3 months after cessation of rHuEpo.

†rhEpo stopped when Hb >14 g/dL and then later resumed.

‡rhEpo stopped because of massive bleeding or no response.

aGVHD = acute graft-vs-host disease; cGVHD = chronic graft-vs-host disease; CRF = chronic renal failure; NA = not achieved; ND = not done. PMF = poor marrow function; PRCA = pure red cell aplasia; sTfR = serum soluble transferrin receptor; TTP = thrombotic thrombocytopenic purpura; Tx = transfusion.

(median 5.5) and peak Hb values from 12.3 to 17.1 g/dL. Maintenance doses of 70 to 350 U/kg/week (median 150) were used. The patient whose response was classified as major was only treated for 10 weeks because she developed grade IV acute GVHD with severe gastrointestinal bleeding and thrombotic thrombocytopenic purpura. Patient 22 with very poor graft function did not respond and remained transfusion dependent. His bone marrow showed complete red cell and platelet aplasia and was not modified after 80 days of rhEpo therapy.

Analysis of erythropoietic response to rhEpo in trials 2 and 3

Figure 1 shows parameters of erythropoiesis and iron metabolism in patients in trials 2 and 3 combined. Baseline Hb, reticulocytes, sTfR, ferritin, and transferrin saturation were similar in the two trials. Soluble TfR increased rapidly (median 3 weeks to double sTfR values) to reach a plateau after 3 to 4 weeks. It slowly decreased to baseline values when rhEpo was discontinued. This expansion of erythropoietic activity translated into more progressive Hb elevation over a period of 10 weeks. Median time to an Hb increment of 2 g/dL was 4 weeks, and it took only 9 weeks to reach an Hb of 13 g/dL. Time to an Hb increment of 2 g/dL was highly correlated with time to reach an Hb of 13 g/dL ($R = 0.95$, $p < 0.0001$) as well as with time to double sTfR levels ($R = 0.85$, $p < 0.0001$). Reticulocytes initially increased but rapidly leveled off (Fig. 1); this was particular true in trial 3 where reticulocytes returned below baseline after 4 weeks in all but two patients, while reticulocyte increases were more sustained in trial 2. Transferrin saturation and ferritin decreased significantly during rhEpo therapy and returned to baseline levels thereafter (Fig. 1); this was particularly systematic in trial 3.

Recovery of erythropoiesis after transplantation in trial 3 was compared with that of historic controls undergoing either PBSC or BM transplant without rhEpo (Fig. 2). Median number of RBC transfusions between days 35 and 100 was 0 in the rhEpo group vs 4 in the PBSC group ($p = 0.0025$) and 10 in the BMT group ($p = 0.0003$). Their transfusion trigger was 8, 9, and 10 g/dL, respectively, explaining differences in Hb levels in the early post-transplant period. Average sTfR levels remained at the lower end of normal values (3000 μg/L) in patients not receiving rhEpo. However, they rapidly increased above the upper normal limit (7000 μg/L) with rhEpo therapy but progressively decreased when the rhEpo dose was reduced. Hb values remained slightly above 10 g/dL in patients not treated with rhEpo, whereas they increased very rapidly in trial 3.

Discussion

Elevated serum Epo levels are observed transiently after intensive conditioning regimens without concomitant changes

Table 4. Response to rhEpo treatment in the third trial

	rhEpo treatment					Hb (g/dL)			RBC transfusions (U/month)				
No.		Starting day after transplant	Starting dose (U/kg/week)	Maximal dose (U/kg/week)	Duration (days)	Baseline	Peak on treatment	Nadir after treatment*	Baseline	Month 1	Month 2	Month 3	Comments
21	29.1	35	500	500	40+§	9.1	12.9	—	4	0	0	—	
22	714.6	35	500	1000	84‡	7.4	Tx	Tx	7	7	8	10	PMF
23	20.3	42	500	500	140	8.5	14.6	12.0	2	0	0	0	
24	34.3	38	500	500	88§	8.2	14.1	Tx	2	0	0	0	Infection, relapse, surgery
25	42.7	49	500	500	133	8.7	13.9	10.0	1	0	0	0	Infection
26	56.8	35	500	500	42†	8.4	13.9	9.9	3	0	0	0	
27	58.1	35	500	1000	80‡	8.1	12.3	Tx	4	0	0	1‡	aGVHD, bleeding, TTP
28	23.8	40	500	500	56	9.4	15.0	10.3	1	0	0	0	Infection
29	32.5	35	500	500	290	8.3	14.3	9.4	3	1	0	0	Infection, bleeding
30	100.6	35	500	500	91†	6.8	17.1	8.4	2	1	0	0	CMV, GVHD, infection
31	21.7	56	500	500	192+	7.8	13.2	—	7	0	0	0	Infection, GVHD, hemorr. cystitis
32	29.5	39	500	500	55§	9.2	14.1	Tx	2	0	0	—	Infection, relapse
33	41.8	38	500	500	172	9.4	14.0	12.0	2	0	0	0	CMV
34	233.4	35	500	500	98	7.6	15.1	10.0	8	0	0	0	Infection
Median	38.1	35	500	500	—	8.4	14.1	—	3	0	0	0	

*During the 3 months after cessation of rhEpo.

†rhEpo stopped when Hb >14 g/dL and then later resumed. ‡rhEpo stopped because of massive bleeding or no response.

§rhEpo stopped at time of death or relapse.

aGVDHD = acute graft-vs-host disease; NA = not achieved; ND = not done; PMF = poor marrow function; sTfR = serum soluble transferrin receptor; TTP = thrombotic thrombocytopenic purpura; Tx = transfusion.

in hemoglobin [3–14]. Peak Epo values usually are observed 7 days after transplant, at the time of the nadir of erythropoietic activity. With marrow recovery, Epo levels progressively return to an appropriate range, and the duration of this correction phase inversely correlates with the speed of engraftment [11]. Thereafter, endogenous Epo remains appropriate for the degree of anemia in autologous transplants [6,7,10–15] but rapidly becomes inadequately low in allogeneic transplants [7–9,13–15]. This defect in Epo production is attributed to the use of cyclosporin A [33,34], which inhibits Epo secretion [35], but acute GVHD [8,13–15] or cytomegalovirus infection [13,15] also contribute. Therefore, the development of erythropoiesis after autologous transplantation is limited by the availability of Epo receptor-bearing erythroid precursors rather than the supply of Epo, whereas after allogeneic HSCT erythropoietic recovery is impaired because Epo levels remain inadequate for prolonged periods of time [14].

Previous trials of rhEpo therapy after allogeneic HSCT have not considered this pathophysiology of erythropoiesis. All studies to date administered very high doses of IV rhEpo starting on day 1 and continuing for 1 to 2 months or until erythroid engraftment. Pilot trials showed accelerated erythropoiesis with increased reticulocyte, sTfR, and/or hematocrit values, as well as a reduction in RBC transfusions compared to historic controls [17,23–26,29,30]. Some even reported an impact on platelet engraftment and/or platelet transfusions [17,25,26,29]. However, larger placebo-controlled studies with rhEpo doses 900 to 1400 U/kg/week confirmed the potential for accelerating red cell but not platelet recovery [18,27,28]. A reduction in RBC transfusions was observed in the smallest trial [28], not in the second one [27] and only between days 20 and 40 (not overall), and particularly in patients with severe acute GVHD in the third trial [18]. Our first trial presented here, even though it consisted of only small numbers of patients, is in line with these findings. Therefore, soaking patients with huge doses of rhEpo at a time when the erythroid marrow has not developed a sufficient number of erythroid precursors to respond and when many intercurrent complications such has organ

toxicity, infection, acute GVHD, and bleeding may blunt response may not be the best way to use rhEpo after an allogeneic transplant.

We took a more physiologic approach in the second trial by providing rhEpo to patients with anemia persisting 56 to 1440 days after transplantation. This proved to be a very efficient strategy, with transfusion independence and complete correction of Hb achieved in 92% and 85% of patients, respectively. A considerable reduction in transfusions already was apparent in the first month of therapy with median doses of 500 U/kg/week, which are 2 to 3 times lower than doses used in previous transplant trials but similar to doses used in cancer-associated anemia. However, the rate and quality of response were far superior to those achieved in the anemia of cancer where the proportion of patients increasing Hb by 2 g/dL is 40 to 60% [36–39]; transfusions are not decreased before the second month [37]; and it typically takes 4 weeks [40] and 6 to 16 weeks [38,39] to increase Hb by 1 and 2 g/dL, respectively. Average maintenance doses were 135 U/kg/week for up to 2 years. We took it a step further in the third trial by scheduling the start of rhEpo at day 35. Responses were even faster, with transfusion independence achieved after 1 week in 90% of patients and complete correction of the anemia after a median of 8 weeks in 80%. With the limitations of differences in type of transplant and transfusion trigger, the transfusion rate was considerably reduced compared to historic controls. Median doses of 150 U/kg/week were used to maintain target Hb values.

The expansion of erythropoietic activity was considerable, reaching sTfR values well above the normal range in the majority of the patients, a feature never observed in allogeneic transplant recipients not receiving rhEpo (Figs. 1 and 2). This expansion reached its maximum even faster than in renal failure patients (who also received lower doses of rhEpo) [41]. When rhEpo therapy was discontinued, erythropoiesis rapidly declined and almost all patients lost several grams of Hb and again became anemic (Tables 3 and 4). This clearly illustrates that post-transplant anemia is Epo dependent and exquisitely responds to substitutive therapy. Hb increases correlated with the expansion of erythropoietic activity and usually occurred rapidly. Other than one patient in both trials 2 and 3 who were not fully evaluable because of severe gastrointestinal bleeding and early death due to acute GVHD, only two patients did not fully respond to rhEpo. Although we did not provide any iron supplements, transferrin saturation rarely decreased below 20% (Fig. 1); thus, functional iron deficiency [42] was not an issue because most patients had very large iron stores from previous transfusions.

Responses were particularly fast when rhEpo was started around day 35 post-transplant. This is not surprising because this is a period when (erythropoietic) marrow recovery is in full motion before cyclosporine-induced Epo deficiency has had enough time to reduce the pool of erythroid precursors

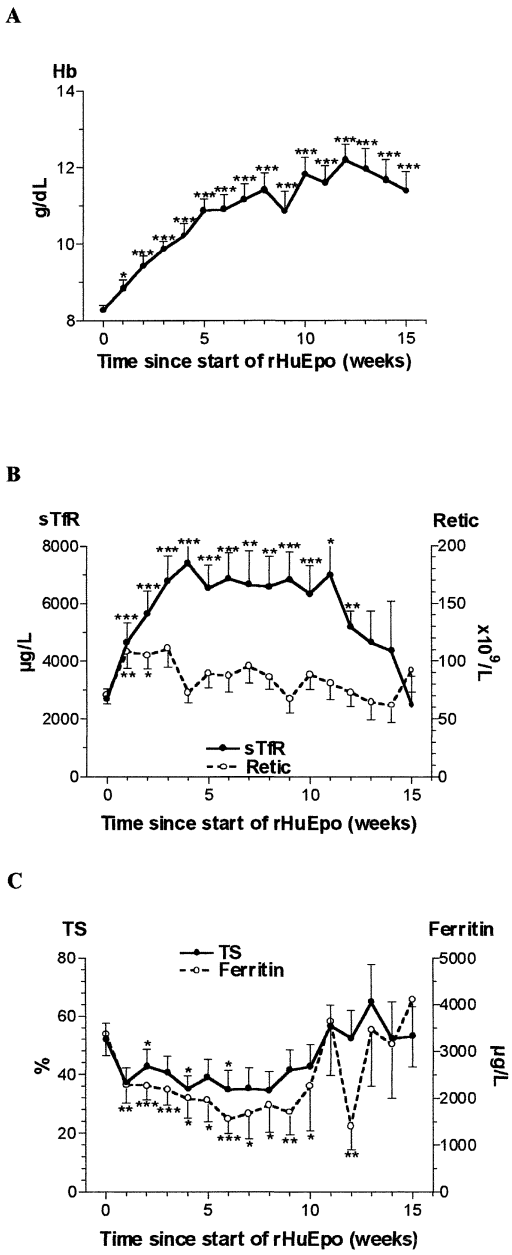

Figure 1. Erythropoietic parameters since start of rHuEpo therapy in trials 2 and 3 combined. The rHuEpo was given for varying periods of time (minimum 5 weeks). TS = transferrin saturation. p Values are given for comparisons with baseline: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

[14]. This timing appears to be optimal because it provides large amounts of Epo just when the erythroid marrow is most susceptible to respond. However, intercurrent complications may blunt or temporarily abort responses; therefore it is preferable to start rhEpo only when major problems such as severe infections and active bleeding or hemolysis are well on the way to resolution.

Our study has the limitations of nonrandomization and small patient numbers. However, the results are impressive and suggest that allogeneic HSCT is associated with the

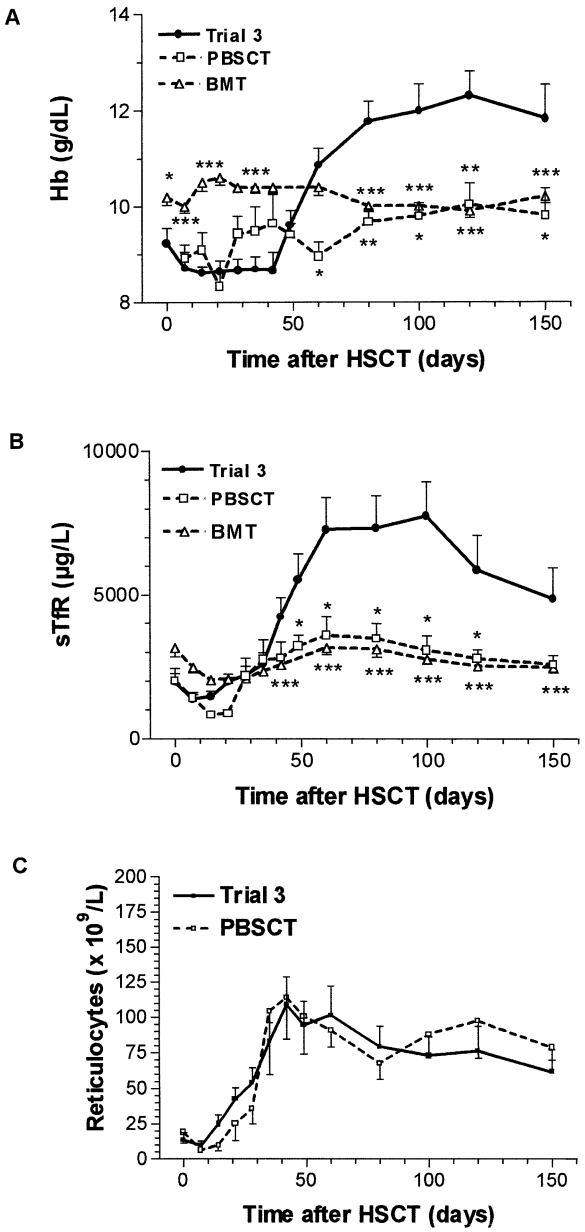

Figure 2. Hemoglobin (Hb), soluble transferrin receptor (sTfR), and reticulocyte counts since day of hematopoietic stem cell transplantation (HSCT) in trial 3 (rHuEpo starting on day 35) compared to historic controls not receiving rHuEpo after a peripheral blood stem cell transplant (PBSCT) or bone marrow transplant (BMT). The initially higher Hb values in historic BMT recipients are due to a higher transfusion trigger. p Values are given for comparisons with trial 3: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

best response rate to rhEpo outside the setting of uremia [43]. Our data set the stage for more rational use of rhEpo after allogeneic HSCT and should renew interest in Epo therapy after the relative disappointment with previous trials targeting initial erythroid recovery rather than the more physiologically appropriate period that follows engraftment. Prospective, randomized, placebo-controlled trials should investigate clinical endpoints previously shown to be improved by rhEpo therapy in other settings, such as transfusion requirements and quality of life.

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